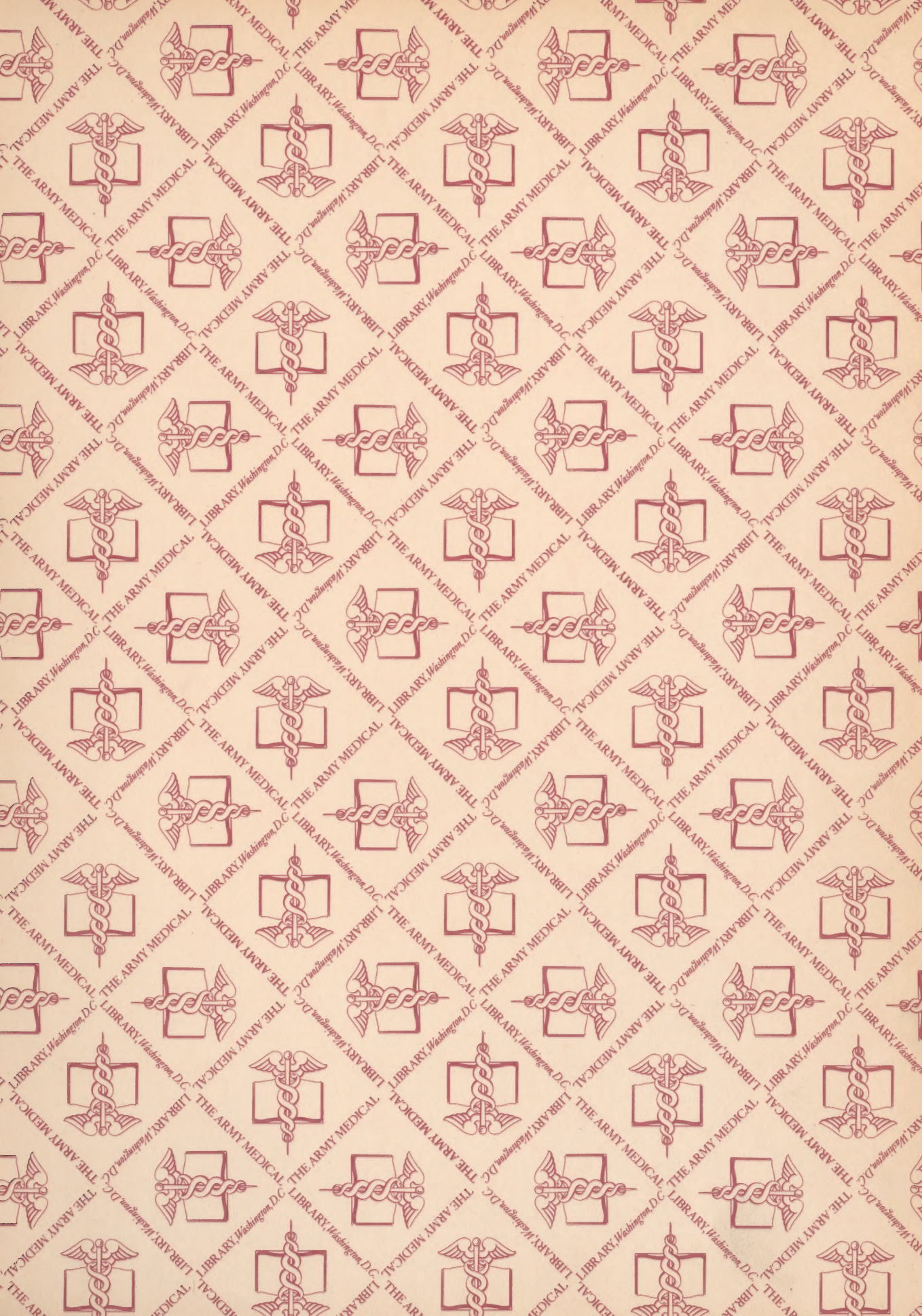

THE ARMY MEDICAL LIBRARY, Washington, D.C.

PARASITOLOGY SYLLABUS

COMPILED AND
PUBLISHED
AT

ENLISTED TECHNICIANS SCHOOL
LETTERMAN GENERAL HOSPITAL

FOR USE OF STUDENTS

PUBLISHED 1941
REPUBLISHED 1942
REPUBLISHED 1943
REPUBLISHED MAY, 1944

TABLE OF CONTENTS

MEDICAL PROTOZOOLOGY

CAPT. WILLIAM S. STONE, M.C.

CHAPTER I

INTRODUCTION

DEFINITION OF MEDICAL PROTOZOOLOGY

Medical Protozoology is the science which deals with those members of the phylum Protozoa that infect man, plus those additional members which may accidentally contaminate the body surfaces, secretions or excretions and may thereby be confused with the truly infectious protozoa.

THE PROTOZOA

The Protozoa are unicellular animals, capable within the limits of that cell of carrying out all the functions necessary for their independent existence and propagation.

I. THE PROTOZOAN CELL

The protozoa are usually microscopic in size and in a given species may vary considerably in this respect, depending upon the suitability of their nutritional environment and upon the stage of their life cycle. The protozoa may be of almost any conceivable shape, and the exact form of the body may be regarded as an adaptation to their mode of life and environment. Their structure varies greatly, but in general, it is made up of a cytoplasmic body and one or more nuclei.

1. CYTOPLASM

The cytoplasm is usually well differentiated into a denser, clear, outer layer, the ectoplasm, and a more fluid, granular, inner layer, the endoplasm.

a. Ectoplasm

The ectoplasm is primarily protective in function and is sometimes very tough and resistant. It may even develop special organelles of defense, i.e., the trichocysts of Paramecia. Certain protozoa have the ability, under adverse environmental conditions, of secreting a very resistant chitinoas-like cyst wall. This cyst wall forms a covering for the resistant stage of the individual and is in direct contact with the ectoplasm.

It is capable of preventing dessication and preserves the life of the organism until conditions are favorable for the resumption of a vegetative existence. Movement, likewise, is a function of the ectoplasm, and in this connection, specialized organelles of locomotion may be formed, e.g., pseudopodia in the amoeba, flagella in trypanosomes, and cilia in Balantidium coli. The capture and ingestion of food is also a function of the ectoplasm. This is accomplished by use of its organelles of locomotion to bring it in contact with its food. It may directly engulf the solid particles at any point on its body surface, as do amoebae, or ingest the particles through special openings (cytostomes) in its surface, as do Balantidium coli. In addition, certain protozoa ingest their food wholly or in part by direct osmosis through the ectoplasm, e.g., malarial plasmodia and trypanosomes. Excretion is also a function of the ectoplasm. This activity is accomplished by special organelles, i.e., contractile vacuoles, cytopyges or anal pores, in some instances, while in others waste material is directly extruded from any point in the ectodermal surface.

b. Endoplasm

The endoplasm carries out the process of digestion and assimilation of the food materials after they have been taken into the cell body. Digestion of solid particles is carried out by the endoplasm forming a digestive vacuole about the ingested particle. The endoplasm then secretes a digestive ferment into the vacuole, which breaks the solid material down into simple products that can be assimilated and used in the anabolism of the cell. In the vegetative organism the endoplasm acts as a circulatory system and is constantly flowing in a more or less circuitous manner. In certain protozoa special supportive structures are formed by the endoplasm, i.e., axostyles in Giardia lamblia.

2. NUCLEUS

The nucleus is usually round or oval in shape when viewed from one plane, but it is actually spherical, elliptical or disc-like in conformation. In size, it is usually 1/10 to 1/6 the diameter of the cell. It is usually fixed in its position within the cell, but in the more primitive forms, it may move with the endoplasmic currents. The nucleus is the center of life in the cell, since when it is injured or destroyed, the cell dies. It is responsible for reproduction, controls growth and reactions to environment, and contains the hereditary determinative factors that perpetuate a given species upon reproduction. The structure and relationship of the component parts of the protozoan nucleus afford the most valuable means of separating and distinguishing closely related species. Therefore, it is of greatest importance to pay close attention to preserving and observing the nuclear structural details. The protozoan nucleus is made up of a clear, achromatic nuclear membrane filled with a semitranslucent nuclear sap. Within the nuclear sap is distributed an intricate, delicate network of linin filaments. Chromatin, a dense, dark staining material, containing the factors of the cell regulating heredity, growth and reproduction is distributed in granules upon the nodal points of the linin network and upon the inner surface of the nuclear membrane. The arrangement and size of these chromatin granules within the nucleus is typical for each individual species. Situated in or near the center of the nucleus is a larger dense staining granule known as the

Karyosome. It differs from the karyosome of the metazoan cell in that it contains, in addition to chromatin, a substance known as plastin. It probably contains the centrosome also.

3. REPRODUCTION.

When dividing, the majority of Protozoa, in some modified fashion or other, undergo mitotic division of the nucleus. However, every possible graduation exists between simple direct division or amitosis (fission) and true mitosis, as seen in the metazoa.

THE METAZOA

In contrast to the protozoa, the metazoa are multicellular animals, the cells of which are differentiated, each performing a special function, i.e., muscle cells contract, bone cells support, gland cells secrete. The cells of the metazoan are also interdependent upon one another for their existence, as is evidenced by the rapid death of any group of cells separated from the remainder of the body.

I. CELL STRUCTURE

The Metazoan cell structure is similar to the protozoan cell with the following exceptions:

1. THE CYTOPLASM

The cytoplasm is not differentiated into ectoplasm and endoplasm. It secured its food and gets rid of its waste products by the process of osmosis with the surrounding media. It does not develop food vacuoles, but certain cells (e.g. Macrophages) may phagocytize solid substances harmful to the metazoan existence. These cells then form vacuoles about the ingested, harmful body and attempt to destroy or neutralize it by secreting destructive ferments into the vacuole. The cytoplasm possesses a definite idiosome or sphere. The centrosome is usually extra nuclear.

2. THE NUCLEUS

The nucleus possesses one or more nucleoli, which have a structure and location similar to that of the karyosome in the protozoan nucleus, but the nucleoli are composed of plastin only. The karyosome of the metazoan nucleus is composed of a few chromatin granules only, and is located upon a nodal point in the linin network.

3. REPRODUCTION

In the metazoa, cell division is always preceded by mitotic division of the nucleus.

The general structures of the Metazoan and Protozoan cells are illustrated in Figure 39.

CLASSIFICATION OF THE PROTOZOA

Classification according to Thomson and Robertson.

Phylum	Protozoa	
Subphylum . .	Plasmodroma	Ciliophora
Class	Sporozoa Rhizopoda Mastigophora	Ciliata

I. SUBPHYLUM PLASMODROMA

This includes the most important organisms which infect man and other animals. This group is distinguished by the fact that syngamy is performed by complete fusion of the male and female gametes, a process known as copulation. In this subphylum there are only 3 classes of medical importance. They are:

1. SPOROZOA

In this class are found the most important protozoan parasites of man, e.g., malaria. The formation of sporozoites occurs after the sexual process of syngamy, e.g., in malaria it occurs in the stomach of the female anopheline mosquito, after which the bite of the mosquito is infective for man.

2. RHIZOPODA

This class is also known as the Sarcodins. They are amoeboid, and movement is produced by means of pseudopodis.

3. MASTIGOPHORA

The organisms included in this class move and feed by the aid of a specially developed ectoplasmic structure, a flagellum, the number of which varies according to the species of flagellates in question.

II. SUBPHYLUM CILIOPHORA

The characteristic feature of this group is the possession of special ectoplasmic structures called cilia. Syngamy is accomplished in this group by juxtaposition of the gametes, followed by exchange of nuclear chromatin. This process is called "conjugation" to distinguish it from the process of copulation in the Plasmodroma.

1. CILIATA

The members of this class have cilia on the surface of their bodies at all stages of development and most of them possess a definite mouth or cytostome.

CHAPTER II

THE SPOROZOA INFECTING MAN.

CLASSIFICATION OF THE SPOROZOA.

CLASS: SPOROZOA.

Subclass	Order	Suborder	Genus	Species
Coccidiomorpha	Coccidiida	Haemosporidiidea	Plasmodium	P. vivax P. falciparum P. malariae Plasmodium in monkeys, birds, etc.
		Eimeriidea	Isospora	I. hominis (I. belli) man
			Eimeria	E. gubleri (?) Man E. perforans) Rabbits E. stiedae) E. clupearum) Fish E. sardinae)

All of the known sporozoan parasites of man are included in the above abbreviated classification. The organisms of this group are characterized by: (1) The asexual cycle takes place by a process known as schizogony, which is usually intracellular, i.e., malaria in the red blood cells, coccidiosis in the epithelial cells of the intestinal mucosa. (2) Male or microgametocytes and female or macrogametocytes are formed in the sexual cycle. Prior to actual sexual union, the female gametocyte becomes a female gamete by extrusion of polar bodies (chromatin), the male gametocyte produces a number of male gametes. There is complete fusion of the male and female gametes.

THE GENUS PLASMODIUM (MALARIA)

The parasites of this genus are pigmented, amoeboid organisms, living on or within the red blood corpuscles of man or some other vertebrate host. The asexual cycle (schizogeny) and the formation of gametocytes (gametogony) are confined entirely to the red blood cells, whereas sporogony is found in the insect vector. In the case of human malaria, man is the intermediate host and the anopheline female mosquito is the definitive host. The definitive host is the one in which the sexual cycle takes place, while the intermediate host is the one in which only the asexual cycle and the development of gametocytes are found. Figure 40 shows the life cycle of the malarial parasite in man and in the mosquito.

The malarial parasites of man are:

I. PLASMODIUM VIVAX (GRASSI AND FELETTI, 1890).

This is the organism of tertian or benign tertian malaria, the most common malarial fever of man. This parasite is distributed geographically over the tropical and subtropical areas, and over certain portions of the temperate zone during the warm months. P. vivax is the hardiest of the malarial parasites and in infected localities in the temperate zone it predominates in the spring and early summer.

1. LIFE CYCLE IN MAN

a. SCHIZOGONY.

The sporozoites are injected into the blood stream by the infected female anopheline mosquito, where they rapidly parasitize red blood cells, round up, and assume the typical signet ring form. These ring forms are about 1/3 to 1/4 the diameter of the infected red cell and when stained by Wright's or Giemsa's method, the chromatin stains red and the cytoplasm blue. In about six hours, the parasite increases in size by one-third and shows marked amoeboid movement within the infected cell. The infected red cell has now enlarged until its diameter is from 10M to 12M and its color is paler than the uninfected red cells. If the infected red cells are properly stained, fine pink dots or stippling (Schuffner's dots) are usually present in the cytoplasm, and minute granules of light brown pigment are seen near the chromatin of the parasite. The growth of the parasite is fairly rapid and by the end of thirty-six hours it fills two-thirds of the enlarged, stippled, parasitized red cell. After about forty hours, the parasite fills the cell and its chromatin usually divides into 12 to 16 segments. Small yellowish brown granules of pigment are now scattered throughout the body of the parasite. The pigment then clumps toward the center of the parasite whose cytoplasm then divides into equal sections, one about each segment of the chromatin. The small bodies resulting from the division of the mature schizont are called "merozoites." At the end of forty-eight hours the merozoites rupture the red cell membrane, (at which time the patient's rigor begins), and then each liberated merozoite seeks out and parasitizes a new red blood cell, to repeat the cycle. The above description depicts the asexual life cycle of P. vivax which, following the time relationship with the rigor, can be demonstrated in the blood stream. At any given time, however, practically all of the stages in the life cycle can be demonstrated in the blood stream, although one stage always predominates. The probable reason for this is that infections in man are due to more than one bite by infected mosquitoes, or that in a given single bite, some of the sporozoites instead of being inoculated intravenously are inoculated subcutaneously, thus delaying the start of their life cycle.

b. GAMETOGONY

The gametocytes or sexual forms develop from certain undifferentiated merozoites. The stimulus for their formation is unknown, but it is probably a response to developing immunity on the part of the host. Four

days are required for the development of the ring forms into mature gametocytes. However, the gametocytes must be from seven to ten days old before they are infectious for the mosquito. When fully grown the gametocytes are rounded in form and have a fairly homogeneous cytoplasm. They appear in the peripheral blood stream within about seven days after the initial fever. Their life in the blood stream is about ten to twenty days. They are probably incapable of reproducing themselves or of initiating the asexual cycle in man without first undergoing sporogony in the anopheline female mosquito.

(1) The male gametocyte (microgametocyte) is 7 M to 8 M in diameter and occupies an enlarged red cell without completely filling it. Schuffner's dots may be present in the margin of the red cell. The cytoplasm of the parasite stains faint blue-grey and contains numerous granules of brown pigment. The chromatin stains red and is scattered over a fairly wide area, sometimes in the shape of a band, near the center or edge of the parasite.

(2) The female gametocyte (macrogametocyte) is larger, 8 M to 10 M, fills the parasitized cell more completely and its cytoplasm stains a darker blue than the male gametocyte. The chromatin of the female gametocyte is in one compact clump, usually near the edge of the parasite, and the pigment is in coarse brown compact granules. There are usually from 3 to 6 female gametocytes for every male gametocyte in the peripheral blood.

2. SPOROGONY IN THE FEMALE ANOPHELINE MOSQUITO

The gametocytes are drawn into the mosquito's stomach along with its blood feed. Within twenty minutes, the male gametocyte develops eight to ten cytoplasmic processes, which soon exhibit violently lashing flagellated ends. These cytoplasmic processes soon separate from the original cell body and are then called "microgametes." While this process has been going on in the male gametocyte, the female gametocyte extrudes two polar bodies containing one-half of its chromatin. It is now ready for fertilization and is called a "macrogamete." The microgamete penetrates the cell wall of the macrogamete, the chromatin of each fuses and the parasite now becomes a motile zygote or ookinete. The ookinete now penetrates the wall of the mosquito's stomach, rounds up, becomes immotile, and is then called an "oocyst." The chromatin of the oocyst undergoes multiple divisions, until there may be as many as 10,000 separate granules, which are grouped together in small clumps. The oocyst now contains from ten to twenty groups of these chromatin granules and is now known as a "sporoblast." Each chromatin granule now develops a fusiform cytoplasmic process and becomes a sporozoite. The sac containing the sporozoites is now called a "sporocyst." The sporocyst ruptures, liberating the motile sporozoites, which soon gain access to the mosquito's salivary glands. Then, if the opportunity presents itself, the mosquito is ready to infect a new individual. Sporogony in the female anopheline mosquito requires about seven to twelve days for its completion, if the temperature is about 20°C. and the air is about 70% saturated with moisture. The life cycle of P. vivax in man showing its relation to the temperature curve of the host is given in Figure 41.

II. PLASMODIUM MALARIAE (LAVERAN, 1881)

This parasite causes quartan malarial fever and it is the rarest of the three species in man. Except for infected transients, it is probably confined to the tropical or subtropical portions of the world. In subtropical regions, the greatest number of new cases of this infection occurs during the fall months. The symptons of this infection are much more severe than would be expected from the number of the parasites infecting the red cells in any given case. In any given infection, all stages are usually present in the blood stream at one time, although one stage always predominates.

1. LIFE CYCLE IN MAN.

a. SCHIZOGONY

Schizogony in Plasmodium malariae is similar to that of P. vivax with the following exceptions: P. malariae requires 72 hours to complete its asexual cycle. During the first 6 hours of its growth it has the largest ring forms of all the types of malaria, and when it is 24 to 48 hours old, it develops typical band forms. Its segmenting forms only develop from 8 to 12 merozoites. The infected red cells are not enlarged and Schuffner's dots are not present. The pigment present is coarse and stains dark brown to black.

b. GAMETOGONY.

Gametogony in Plasmodium malariae is similar to that in P. vivax, except that the organisms are smaller and the red cells are not enlarged, and do not contain Schuffner's dots. The time required for its gametocytes to develop is approximately seven days.

2. SPOROGONY

The life cycle in the female anopheline mosquito is the same as for P. vivax, except that it takes about three times as long to complete the cycle. The life cycle of P. malariae in relation to the temperature curve of the host is shown in Figure 42.

III. PLASMODIUM FALCIPARUM (WELCH, 1897)

This is the organism of estivo-autumnal, subtertian, malignant tertian or pernicious malaria. Its distribution is similar to that of Plasmodium malariae, but it is second only to P. vivax in the frequency of its occurrence. It is the most severe of all the malarial fevers of man and requires prompt early diagnosis and treatment.

1. LIFE CYCLE IN MAN.

a. SCHIZOGONY

Schizogony is similar to that of P. vivax with the following exceptions: (1) The ring forms are smaller when they first parasitize a red blood cell. (2) There are more apt to be multiple infections of the red cells and ring forms may show two dots of chromatin. (3) The ring stage may persist for about 24 hours in the peripherial circulation during which time it doubles or triples its size. (4) Ring forms and mature gametocytes (crescents) ordinarily are the only forms found in the peripheral blood. In severe, overwhelming infections by this parasite bizarre filamentous and other forms may occasionally be present in the peripheral blood. (5) Developing schizonts and gametocytes are usually found only in the capillaries of the internal organs. These organisms have a marked tendency to clump together and stick to the endothelial cells, thus bringing about capillary blockage. (6) Segmenting forms of P. falciparum develop 16 to 32 merozoites. (7) The parasitized red blood cells are not enlarged. (8) Schuffner's dots are not present in the parasitized cells, but large granules staining purplish red, called "Maurer's dots" or malignant stippling are present.

b. GAMETOGONY

The gametocytes are crescent-shaped with rounded ends and they are greater in length than the diameter of the red cells which they have parasitized. The male gametocyte stains bluish-gray and has a central nucleus with diffuse chromatin. The pigment present is in coarse grains and stains brownish-black. It is scattered between the grains of chromatin. The tips of the male crescent are usually more rounded than those of the female crescent. The female gametocyte (crescent) stains sky-blue and its nucleus is made up of a compact mass of chromatin and pigment.

2. SPOROGONY

The life cycle of P. falciparum in the female anopheline mosquito is essentially the same as that of P. vivax, except that 1/3 to 1/2 again as much time is needed to complete the cycle. Figure 43 gives the life cycle of P. falciparum in relation to the temperature curve.

IV. DOUBTFUL SPECIES AND SUBSPECIES OF MALARIAL PARASITES OF MAN.

The doubtful species and subspecies of the malarial parasites of man are:

1. PLASMODIUM FALCIPARUM QUOTIDIANUM. (Craig, 1909)

This organism is differentiated from P. falciparum infections clinically by more or less constant fever with daily exacerbations. The ring forms of the parasite are smaller than those of P. falciparum and only 12 to 14 merozoites are produced at segmentation.

2. PLASMODIUM OVALE (Stephens, 1922)

This organism is identical with P. malariae, except for the absence of band forms. In addition all parasitized red blood cels are oval in shape.

3. PLASMODIUM VIVAX, VARIETY MINUTUM (Emin, 1914)

This organism is identical with P. vivax except that the parasites never enlarge the parasitized red cells. The schizonts only fill two-thirds of the parasitized red cell and only 4 to 10 merozoites are produced at segmentation. Table 85 presents the characteristics differentiating the three main species.

V. PLASMODIA OF MONKEYS

These organisms are identical morphologically, in general terms, with those of man, but repeated attempts to transmit them to man and to infect monkeys with human malaria have been unsuccessful insofar as maintaining the organisms through successive transfer is concerned.

VI. THE EXAMINATION OF MOSQUITOES FOR MALARIA

Various surveys in heavily infected areas in different parts of the world seldom reveal infection of more than 0.2% to 2% of the chief anopheline vector. In tropical countries all of the anopheline mosquitoes found infected with malarial parasites are not necessarily human malarial vectors, for the parasites present may be those of monkey malaria which is morphologically identical with that of human malaria, and a differentiation between the two can only be made by transmission to man or the species of monkeys present in the area. Therefore, in mosquito surveys, to determine the malarial vectors of any given locality in which malarial fever is present either in an epidemic or endemic form, great care should be taken to examine a sufficient number of the suspected species of anophelines to draw an accurate conclusion. As a routine at least 100 to 400 females of a given species should be examined. The procedure for examining them for the presence of malarial infection is as follows:

1. COLLECTION OF MOSQUITOES.

Capture the anopheline mosquitoes near human habitation and preferably near the houses of known cases or carriers. Keep the mosquitoes alive until the time of examination.

2. KILLING MOSQUITOES FOR EXAMINATION.

Place four to six mosquitoes in a glass jar containing a small amount of cotton moistened with chloroform. As soon as they are dead remove them from the jar and place between layers of cotton moistened with water until they are dissected.

3. TECHNIQUE OF DISSECTION.

a. Materials required

(1). Clean slides and cover glasses.
(2). Two straight dissecting needles in needle holders, one to have a two-edged cutting point.
(3). Pipettes or droppers.
(4). A pair of fine pointed scissors to clip off the wings and legs.
(5). A pair of fine pointed tissue forceps to handle the dead mosquito.
(6). Alcohol lamp.
(7). Physiological saline solution.
(8). Physiological saline solution deeply tinted with methylene blue.

b. Precautions.

(1). Mosquitoes should be dissected within one hour after they have been killed as autodigestion of tissue rapidly sets in and it will be almost impossible to secure proper specimens if more than two or three hours are allowed to elapse before beginning the dissection.

(2). Never let the preparation become dry while dissection is in progress; always keep the specimen in a drop of physiological saline.

(3). When the alimentary canal is removed be careful not to break up the malpighian tubules as granules liberated by so treating them will greatly confuse the picture.

c. Procedure.

(1). Select one of the dead female anopheline mosquitoes, identify its species, record the results and then remove the wings and legs. Place the body on a glass slide in a drop of saline solution tinted with methylene blue so that its ventral surface is toward you and the tip of the abdomen points to your right.

(2). The salivary glands lie in the forequarter of the thorax; to remove them, hold the body in place by gently pressing the tip of a dissecting needle on the anterior half of the thorax, then with the shaft of the second needle press on the head, gently drawing it away from the thorax. By this procedure the pair of very minute, blue-stained, refractile, trilobate glands are usually extracted attached to the head. If the glands fail to come away with the head, they may sometimes be expelled by gentle pressure with a dissecting needle on the anterior and middle portions of the thorax, or as a last resort by teasing the thorax to shreds. Remove the glands from the head by means of the cutting-edged dissecting needle, then by securing them on the needle tip, transfer them to a small drop of unstained saline on another slide and cover with a cover glass.

(3). To obtain the stomach, place the tip of one needle on the 7th and the other on the 8th abdominal segments, break the chitinous connection between these segments by gentle teasing, then transfix the thorax with one of the needles and pierce the last segment of the abdomen with the other; now using gentle traction on the last abdominal segment, draw out the viscera into the saline solution. Secure the stomach by means of the shaft of the needle in the left hand, then with the right hand, using the needle with the cutting edge, trim off the malpighian tubules and all structures lying posterior to them. Now pick up the stomach on a needle tip, transfer it to a large drop of untinted saline on a clean slide, straighten it out and cover with a cover glass. Withdraw enough of the saline from the preparation by means of a slip of filter paper so that the cover glass no longer floats, then gently heat the slide over the alcohol lamp until the stomach becomes distended due to the heat expansion of its gaseous content.

4. EXAMINATION OF THE SPECIMEN

a. The Stomach.

Center the stomach with the long axis perpendicular to the observer, in a low-powered microscopic field, then place the point of a dissecting needle against the left edge of the cover glass and hold it stationary. Now move the slide to the left by means of the mechanical stage, which will cause the stomach to revolve and in its revolution all its external surface can be examined for projecting cysts. All suspicious spherical bodies found attached to the stomach wall should be examined under higher magnification. The species of large cysts can not be recognized, but in small and medium sized cysts, the pigment is fairly characteristic. P. falciparum cysts have dense clumps of large, black, pigmented granules and P. vivax cysts have delicate strands of yellow to brown, fine-pigmented granules, while P. malariae cysts have clumps of coarse, dark-brown, pigmented granules. For the exact morphology of malarial parasites in the wall of the mosquito stomach see Figure 44.

b. The Salivary Glands

Place the point of a dissecting needle on the cover glass directly over the salivary glands, then by gentle repeated pressure crush the glands. The crushed glands and adjacent fluid are then examined under the high power or oil immersion objective for malarial sporozoites. The species of sporozoites can not be determined from their size or morphology. If in doubt about the presence of sporozoites, the cover glass should be removed, the preparation allowed to dry, after which it is stained by the Wright or Giemsa method. Examination of the stained smear will establish whether or not sporozoites are present.

VII. LABORATORY DIAGNOSIS

For the laboratory diagnosis of malaria see Laboratory Methods, Chapter V.

THE COCCIDIA

The coccidia are sporozoon parasites of the lining cells of the intestine of vertebrates. One species, Eimeria stiedae, parasitizes the cells lining the bile ducts of the liver. For a typical life cycle in this group see Figure 45.

There are only two species of coccidia that parasitize man. They are:

I. ISOSPORA HOMINIS (I. BELLI).

This infection in man is found throughout the tropical and subtropical world, but the majority of cases reported to date have arisen in countries around the eastern end of the Mediterranean. There have been more than 150 cases of this infection recorded. This infection is characterized by abdominal discomfort for 3 to 5 days followed by diarrhea with considerable tenesmus, which persists for 3 to 6 weeks. The patient, although greatly debilitated, usually recovers. After about the third week of the diarrhea oocysts are passed in the stools. They are covered by a doubly refractive wall, are ovoid in form and measure 15 x 30 M. The cyst is smooth, colorless and very resistant to fixatives. A micropile at one end of the cyst can sometimes by made out. The oocyst when passed, contains a single ball of cytoplasm. This soon divides into two masses which secrete a wall about themselves. Each cytoplasmic mass then divides into four sausage-shaped sporozoites. This cycle requires about 2 days for its completion, at which time the sporocysts are infective if again ingested. The oocysts continue to be passed during the period of diarrhea, although they are never in greater numbers than two or three per slide. They are graphically illustrated in Figure 46.

II. EIMERIA GUBLERI (?)

This is a coccidial infection of man involving the epithelium of the bile ducts in the liver. There have been only a few cases recorded in medical literature and this parasite has never been thoroughly studied.

III. EIMERIA CLUPEARUM AND E. SARDINAE

These organisms cause coccidiosis in various species of fish. When fish infected with these organisms are ingested by man, the oocysts of the parasites are liberated in the gastro-intestinal tract, but are unable to infect the epithelium and are eliminated in the feces. Therefore, finding the oocysts of these species in the feces is of no significance.

CHAPTER III

RHIZOPODA (AMOEBAE)

CLASSIFICATION

CLASS: RHIZOPODA

ORDER	GENERA	SPECIES	HABITAT
Amoebida	Entamoeba	E. coli	G. I. tract man
		E. histolytica	" " "
		E. gingivalis	Buccal cavity man
	Endolimax	E. nana	G. I. tract man
	Iodamoeba	I. butschlii	" " "
	Dientamoeba	D. fragilis	" " "
	Suppinia	S. diploidea	Coprozoic
	Dimastigamoeba	D. gruberi	"
	Amoeba	A. proteus	Free living

The amoebae infecting man show fairly marked differentiation of the cytoplasm into a clear, hyaline outer zone or ectoplasm and a granular inner zone or endoplasm. They move by means of pseudopodia and, with the exception of E. gingivalis and D. fragilis, they are transmitted from infected to new cases in the form of resistant cysts.

The amoebae of medical importance may be conveniently grouped into the pathogenic amoebae, the commensal amoebae, and the confusing contaminating coprozoic and free living amoebae.

THE PATHOGENIC AMOEBA OF MAN

I. ENTAMOEBA HISTOLYTICA (SCHAUDINN, 1903)

E. histolytica is the only known pathogenic amoeba of man. It is now recognized that it is universal in its distribution and while infection is most prevalent in the tropical and subtropical regions, it is by no means uncommon in the temperate zone. It is estimated that from two to ten percent of the population of the United States harbor this parasite. It is the organism causing amoebic dysentery and its sequellae in man.

1. LIFE CYCLE

The stages of the life cycle of E. histolytica may be divided into the free vegetative forms or trophozoites, the precystic and the cystic forms.

a. Trophozoites.

The trophozoites of E. histolytica vary in size, depending upon the suitability of their environment and the rapidity of their multiplication. At times, in any given specimen or culture, it is possible to demonstrate vegetative forms of E. histolytica in sizes varying from 8 to 40 M. The average size, when rounded up, is about 25 M in diameter. The ectoplasm is very well differentiated from the endoplasm; this is particularly true when the amoeba is not moving, but sending out a pseudopod and then withdrawing it to send out another. Under these conditions it is glassy clear. When the amoeba is rapidly moving, however, there is only moderate differentiation of the ecto- and endoplasm. The endoplasm is finely granular and has very little color. Although E. histolytica will ingest bacteria, starch and other kinds of food in culture, in the body it is a true tissue parasite and lives on body cells and fluids to the exclusion of all other types of food. In the fresh diarrheal stool, E. histolytica very rarely contains ingested bacteria in its endoplasm. Under these conditions, the only ingested solid particles are red blood cells or leucocyte fragments. Except in individuals undergoing degeneration, the only vacuoles present in the endoplasm are food vacuoles. The nucleus is about 1/6 the diameter of the cell, is disc-like in shape, and moves with the endoplasmic currents, occasionally tilting slightly as it floats along. In the active living trophozoite it is sometimes very difficult to visualize, but as it is usually in that portion of the endoplasm that flows into the new-formed pseudopod, it can be observed in this position contrasted against the ectoplasm. When the nucleus is stained with iodine or hemotoxylin the chromatin is seen to be distributed in fine fairly even granules about the inner side of the nuclear membrane. The linin net work is free from chromatin and the karyosome is a small centrally located granule. Reproduction is by binary fission, which can be easily observed in warm stage preparations from rapidly growing cultures. It is usually an equal division of nuclear and cytoplasmic substances, but in rapidly growing specimens there may be marked unequal divisions of the cytoplasm, thus giving rise to the small organisms which were formerly thought to be a different strain. When not undergoing division, precystic changes, or in unfavorable environment, the vegetative E. histolytica moves rapidly, progressively, and more or less continuously. The organism moves like a slug, and has the ability to cling to a surface and make progress even against or across currents in the surrounding media. The movement of amoebae, against currents or solid particles in the media, is accomplished by extending a pseudopod as far as possible, and then after fixing the tip of this pseudopod on a solid surface, the amoeba will push out a new pseudopod from a point near the tip of the previous pseudopod which is now used as a fixing point. Thus, with one stationary point, the amoeba is able to push out the new pseudopod and allow the remainder of its body to flow into it until the tip of the first pseudopod is the rear most part of the amoeba. The process is then repeated. Moving E. histolytica usually have considerable debris trailing along behind each one.

b. Precystic stage.

When the environmental conditions gradually become adverse

for its continued vegetative existence, E. histolytica rounds up, becomes practically immotile and extrudes all undigested solid food particles. The chromatin granules in the nucleus become coarser and the nucleus is now quite visible in the fresh state when viewed under high power. Rod-shaped chromatoid bodies are sometimes formed in the later part of this stage when the ectoplasm is about to secrete a cyst wall. This stage is known as the precystic stage, but when the adverse environmental changes are sudden, the amoeba is uable to undergo this stage and merely rounds up and dies.

c. Cystic Stage.

Following the pre-cystic stage a definite, clear, hyaline cyst wall is secreted by the ectoplasm. Cysts are not formed in the tissue of the host; they are present only in fecal excrement and in cultures. The cysts vary in size from 5 M to 20 M and their cytoplasm is clear, translucent and finely granular. In about 20% of the new-formed cysts there is a large refractile red-shaped body with rounded ends, the chromatoid body. This body and its shape are characteristic for E. histolytica, and if it can be observed in a cyst, a diagnosis of E. histolytica can be made. When present, the chromatoid body can be visualized by reduced light in the fresh unstained state. It stains a bluish-black with hematoxylin, but it is not visible in iodine preparations. The iodine stain may show, in new-formed cysts, small glycogen vacuoles that are light mahogany in color. Both the glycogen vacuoles and chromatoid bodies gradually disappear as the cysts age. When the cyst is first formed, there is only one nucleus present, and it is of the same type as that in the vegetative forms. This nucleus soon divides, then each daughter nucleus divides again giving a total of four small characteristically shaped nuclei, each lying on a different plane in the spherical cyst. The average cyst contains four nuclei and it is this four nucleated cyst with or without the red-shaped chromatoid body that is characteristic for this species. The cysts, upon being passed in the feces, are fairly resistant to mild dessication and ordinary disinfectants (ordinary water chlorination will not kill them). In the feces as passed, cysts will probably live not longer than from 4 to 10 days. However, if the fecal matter is diluted with water, as in sewage, and the temperature does not exceed 20°C., they may be viable for periods up to 200 days. They are destroyed by heating to 50°C., for five minutes. The cyst is the only state in which amoebae can pass through the gastric juices and still remain viable. For this reason, contamination of food, drink or mouth parts by cysts of E. histolytica is the only natural way that this infection can be acquired. In addition to the ordinary means of contamination, flies and cockroaches may ingest and pass viable cysts in their fecal excrement.

When the cysts of E. histolytica are ingested, they pass unharmed down the intestinal tract until they reach the cecum. The ex-cystation takes place and the four amoebae resulting from each cyst seek a tubular gland where they propagate and soon destroy enough of the cells of the host to plug the gland. The small pin-point yellow abscess so formed is now freed of most of the bacteria and the amoebae digest away the tissue cells and undermine the mucosa. They invade the surrounding lymph spaces,

the connective tissue planes, and thrombose the neighboring capillaries. The spreading necrotic area thus formed soon sloughs out and leaves a small undermined ulcer. The amoebae on the surface of this ulcer are washed out into the intestinal contents by the fecal currents, where they either invade new glands, become encysted, or are passed as trophozoites, thus completing the cycle. If the host resistance is poor, new ulcers will be formed in rapidly increasing numbers, and the individual will become aware of gaseous distension and vague abdominal discomfiture, which is soon followed by symptoms of dysentery. If the host's resistance is good, only a limited number of ulcers will be formed and then a chronic course ensues (known as the carrier state). However, if the host's resistance is lowered, it may at any time become an acute case. Liver abscess is stated to occur in about 15% of all untreated cases infected with E. histolytica.

THE COMMENSAL AMOEBAE OF MAN

The commensal amoebae of man are of importance in that they may be confused with E. histolytica, and because their presence indicates human fecal contamination at some time of the mouth parts, fluids, or food ingested by the infected individual.

I. ENTAMOEBA COLI (GROSSI, 1879) CASACRANDI AND BARBAGALLO, 1895.

E. coli is a harmless saprophyte living in the lumen of the human large intestine. This is the most common amoebic infection of man's gastro-intestinal tract and is the one most easily confused with E. histolytica.

1. THE LIFE CYCLE OF E. COLI

The life cycle of E. coli is very similar to that of E. histolytica with the following exceptions: (1) E. coli is not pathogenic. (2) Its cytoplasm is coarsely granular and is not as well differentiated into ecto- and endoplasm. (3) It rarely ingests red cells and is usually filled with bacteria, starch granules, mold spores, etc. (4) Its nucleus is easily visible and contains coarse irregular-sized grains of chromatin on the inner surface of the nuclear membrane. (5) The linin network may have a few grains of chromatin suspended in it, and the karysome is larger and eccentrically located in the nucleus. (6) E. coli moves sluggishly and usually does not make a great deal of progress, and it is very easily kept in the microscopic field as compared to E. histolytica. (7) The average cyst of E. coli usually has 8 small fairly typical nuclei. The chromatoid bodies when present have sharp pointed acicular ends (demonstrated by careful focusing) as compared to the rounded ends of E. histolytica. (8) When first formed, the cysts usually contain larger and more marked glycogen vacuoles.

II. ENTAMOEBA GINGIVALIS (GROS, 1894) Brumpt, 1913.

Entamoeba gingivalis is a harmless saprophytic amoeba found in the pus pockets of pyorrhea alveolaris, tartar deposits, cavities of carious teeth and in any buccal erosion or abscess.

1. LIFE CYCLE

The life cycle of E. gingivalis is confined to the buccal cavity of man, and is the only amoeba living in this location. The trophozoites are almost identical in appearance with those of E. histolytica except that they are usually filled with dark pyknotic bodies and are more sluggish in their movements. Cysts have never been demonstrated for this amoeba. Animals susceptible to infection with E. histolytica, when inoculated per rectum with E. gingivalis, have never revealed any pathogenicity in this species.

III. ENDOLIMAX NANA - (WENYON AND O'CONNOR, 1917) BURG, 1918

E. nana is a small non-pathogenic amoeba which inhabits the lumen of the human colon. Next to E. coli this is the most common amoebic infection in man's gastro-intestinal tract. This amoeba is of no importance clinically, except that it may be confused with small strains of E. histolytica.

1. LIFE CYCLE

This organism is the smallest amoeba infecting man, measuring from 6 M to 12 M. The life cycle is similar to E. histolytica with the following exceptions: (1) It is a slow moving, sluggish organism showing more changes in shape than progressive motion. (2) All the chromatin of the nucleus is collected into a large irregularly-shaped karyosome which is usually eccentrically located in the nucleus. (3) There is no chromatin on the inner surface of the nuclear membrane. (4) The cysts are oval or irregular in shape and usually contain 4 small nuclei similar to those present in the trophozoite. (5) There are no chromatoid bodies present in the cyst.

IV. IODAMOEBA BUTSCHLII - (PROWAZEK, 1912) DOBELL, 1919.

I. bitschlii is one of the rarer non-pathogenic amoeba inhabiting man's colon.

1. LIFE CYCLE.

This organism usually varies from 9 M to 13 M in size. Individuals infected with this organism usually pass many irregularly-shaped cysts, but rarely trophozoites in their feces. The trophozoites resemble E. coli in form and habit, except that the nucleus has a large oval or round eccentrically located karyosome containing all the chromatin. The cysts are very irregular in shape and contain either one or two typical nuclei. Their most characteristic feature is that, when they are stained with Gram's iodine (double strength) large, deeply staining, mahogany brown glycogen vacuoles are found to be present. These glycogen vacuoles may nearly fill the cyst and displace the nuclei to one side. The glycogen bodies get smaller with age and may eventually almost disappear.

V. DIENTAMOEBA FRAGILIS - JEPPS AND DOBELL, 1918.

This is the rarest intestinal amoeba of man, and is a harmless saprophyte inhabiting the colon. It is extremely sensitive to changes in environment and rapidly degenerates after being passed in the feces.

1. LIFE CYCLE

This organism ranges from 3 M to 12 M in size. The endoplasm is finely granular and the ectoplasm well differentiated. This organism usually does not move, but merely changes shape by extruding pseudopods which, when fully extended, spread laterally. The majority of this species have two nuclei; in fact, the uninucleated amoeba are out numbered four to one. The chromatin is arranged in a ring of from 4 to 6 granules located in the nucleus halfway between the central point and the nuclear membrane. There is no chromatin on the nuclear membrane. Cysts have never been demonstrated for this species.

DIFFERENTIAL DIAGNOSIS

The principle points of differentiation between the various species of amoebae infecting man are illustrated in Figure 47 and listed in Table 86.

THE COPROZOIC AND FREE LIVING AMOEBAE

I. THE COPROZOIC AND FREE LIVING AMOEBAE, that may contaminate the body secretions or excretions and thus may be confused with the amoebae that truly inhabit the mouth or gastro-intestinal tract of man, are characterized by the possession of at least one contractile vacuole, e.g., Amoeba protoùs. This structure is never present in the amoebae infecting man.

THE LABORATORY DIAGNOSIS

I. THE LABORATORY DIAGNOSIS of these organisms is accomplished by: (a) Direct microscopic examination of the fresh excretions or secretions. (b) Preparation and examination of stained specimens. (c) Cultures. (d) Differentiation from confusing objects. The exact methods in performing these procedures are given in Laboratory Methods, Chapter V.

CHAPTER IV

THE MASTIGOPHORA AND CILIATA INFECTING MAN.

CLASSIFICATION

CLASS: MASTIGOPHORA (FLAGELLATES)

Subclass	Order	Genus	Species	Habitat
Phytomastigina	Euglenoidida	Copromonas	C. subtilis	Coprozoic
Zoomastigina	Protomonadida	Embadomonas	E. intestinalis	Infests mucous surfaces of man
		Tricercomonas	T. intestinalis	Infests mucous surfaces of man
		Chilomastix	C. meanili	Infests mucous surfaces of man
		Trichomonas	T. hominis	Infests mucous surfaces of man
		Bodo	B. caudatus	Coprozoic
		Leishmania	L. donovani	Blood stream and tissues of man
			L. tropica	Blood stream and tissues of man
		Trypanosoma	T. gambiense	Blood stream and tissues of man
			T. rhodesiense	Blood stream and tissues of man
			T. cruzi	Blood stream and tissues of man
	Diplomonadida	Giardia	G. lamblia	Infest small intestine of man
CLASS CILIATA (CILIATES)				
Spirigera	Heterotrichida	Balantidium	B. coli	Infects large intestine of pig and man.

THE MASTIGOPHORA (FLAGELLATES)

The organisms of the class Mastigophora are characterized during the major part of their existence by the possession of single or multiple, long hair-like processes called flagella, which they use for locomotion in the liquid media of their habitat. The flagellates may be conveniently divided

into the following groups: (1) Those saprophytic upon the mucous surfaces of man; (2) Those contaminating the excretions of man after they have been eliminated from the body (coprozoic flagellates); (3) Those infecting the blood stream and tissues of man.

I. THE SAPROPHYTIC FLAGELLATES OF THE MUCOUS SURFACES OF MAN.

1. Chilomastix mesnili- (Wenyon) Alexeieff, 1910. This organism is probably universal in its distribution. It is a harmless saprophyte infesting the lumen of the large intestine of approximately four percent of the world's human population. It is a flexible, pear-shaped organism, 10 M to 15 M in diameter, with the small, posterior end tapering into a fine caudal process or tail. The large, rounded, anterior end is penetrated by a spiral groove which extends about one-third the distance to the caudal extremity. At the bottom of this groove is found the cytostome. The nucleus is located in the anterior end and is round or oval in shape. Its chromatin is often clumped on one section of the inner surface of the nuclear membrane. The blepharoplasts are in apposition with the most anterior portion of the nuclear membrane and give rise to three anterior free flagella and one flagellum directed backwards and attached to the edge of the spiral groove. The movement of this flagellate is characteristic. It moves in a slow deliberate manner with slight oscillation in its direction and slow rotation of its body. Its transmission to new hosts is accomplished by contamination of food or water with resistant cysts passed in the feces of individuals harboring this infestation. The cysts are shaped like a watermelon seed and they are about 10 M in size. For a graphic representation of the morphology of this organism, see figure 48.

2. Trichomonas hominis - Davaine, 1860. Synonyms: T. intestinalis, T. vaginalis, T. elongata and T. buccalis. This is the most common saprophytic flagellate of man. The concensus of opinion is that it is a harmless saprophyte. It may infest the buccal cavity, the lumen of the colon or the lower portion of the genito-urinary tract. T. hominis varies from 5 M to 20 M in size and is round or oval in shape. The nucleus is located in the anterior end and there are from three to five free flagella. One flagellum is directed backwards and is attached to the free margin of a linear undulating membrane which runs the full length of the organism. A special, clear, elastic, rod-like supporting structure (called the axostyle) runs longitudinally through the center of the organism and may extend beyond the posterior body limits forming a posterior spine. This flagellate has a fast nervous type of movement and is constantly darting about. The body rotates rather rapidly and under oil immersion with reduced light or on darkfield examination, the undulating membrane is clearly visible. Cysts have never been demonstrated for this organism. See figure 49 for a graphic representation of the morphology of this organism.

3. Giardia lamblia - Stiles, 1915. This is the third most common saprophytic flagellate of man. It inhabits the lumen of the upper portion of the small intestine and free living forms are never present in the stools except in the presence of a diarrhea. There is still considerable controversy prevalent in regard to the pathogenicity of this organism, but it is probably a harmless saprophyte. G. lamblia is pear-shaped, tapering to a point at the small posterior end, and it is about 7 M by 14 M in size. The anterior portion of the ventral surface is concave and acts as a sucking disc for attachment to the intestinal mucosa. G. lamblia is bilaterally symmetrical, all the organelles and other structures are paired or lie equally on both sides of the middle line. There are two oval, vesicular-shaped nuclei with central karyosomes which are sometimes rod-shaped or are in separate granules. The nuclei lie in the anterior rounded portion of the organism, dorsal to the ventral sucker and are symmetrically located so that one nucleus is on each side of the mid-line. Two blepharoplasts are situated near the mid-line of the body just anterior and mesial to the nuclei. Two axostyles (strengthening rods) arise from the blepharoplasts and pass backward to the caudal end of the organism. Four pairs of flagella arise along the mid-line of the ventral surface, the anterior pair cross each other before emergence from the cytoplasm. A dark-staining, comma-shaped rod sometimes lies across the posterior part of the axostyles.

The cysts are oval in shape, about 7 M by 10 M in size, and contain two to four nuclei and rudimentary axostyles plus comma-shaped bodies. There may be considerable variation in the content and relationship of the internal structures. See figure 50 for a graphic representation of the morphology of this organism.

4. Embadomonas intestinalis - Wenyon and O'Connor, 1917. This is a rare, non-pathogenic intestinal flagellate that has occasionally been found in human feces. It is a small (4 M by 9 M) very actively motile flagellate, pyriform in shape and when viewed from the side is said by some to be slipper-like in appearance. The cysts are very small (2.5 M by 7 M) and are difficult to find. For morphology see figure 51.

5. Tricercomonas intestinalis - Wenyon and O'Connor, 1917. This is a rare, non-pathogenic intestinal flagellate of man found mostly in the Federal Malay States. It infests the lumen of the colon and is about 4 M by 10 M in size. It is an extremely active flagellate with a very plastic, almost amoeboid body. For morphology see figure 51, above.

COPROZOIC FLAGELLATES

The coprozoic flagellates are free living organisms that may accidentally contaminate fecal specimens after they have been passed. They usually possess contractile vacuoles and nuclei with a very large, centrally located karyosome containing practically all of the chromatin. Bodo caudatus and Copromonas subtilis are the two most frequently encountered organisms in this group. For their morphology see figure 52.

THE FLAGELLATES INFECTING THE BLOOD AND TISSUE OF MAN

The flagellates infecting the blood and tissue of man can be divided into the Leishmania and the Trypanosomas. These flagellates undergo morphological changes during certain stages of their life cycle and in discussing the life histories of these parasites, it will be necessary to refer to a given stage by the name designated for it. For the sake of clarity, definitions of each stage are given below, and are illustrated in figure 53.

(1) Leishmania. The body is round or oval and the kinetoplast, which is made up of two structures, the parabasal body and the blepharoplast, lies near the centrally located nucleus. There is no free flagellum or undulating membrane.

(2) Leptomonas. The body is elongated oval in shape with the nucleus in the center and the kinetoplast in the anterior tip. There is a free flagellum, but no undulating membrane.

(3) Crithidia. The body is identical with that of the leptomonas except that the kinetoplast is almost in apposition with the anterior surface of the nucleus. There is a short undulating membrane between the kinetoplast and the anterior tip of the organism. The flagellum runs from the blepharoplast along the free edge of the undulating membrane and may or may not become free at the anterior end.

(4) Trypanosome. This is the same as the crithidia form, except that the kinetoplast is in the posterior tip of the organism and there is an undulating membrane running the full length of the body. The flagellum may or may not have a free anterior end.

(5) Metacyclic forms. In insect vectors the trypanosomes undergo a cyclic development. The organisms that occur at the end of the cycle are identical with the blood trypanosomes, except that they are smaller.

Polymorphism or monomorphism in referring to trypanosomes does not mean variation in shape or size of the body, but indicate whether or not forms in the blood stream that are not undergoing division are uniform in regard to the type of flagellum they possess. Thus, if both free and bound forms of flagella are present they are polymorphic, and if only one form is present, they are monomorphic.

Multiplication is by means of longitudinal fission. The kinetoplast divides first, then the nucleus, and lastly the cytoplasm.

I. LEISHMANIA

These organisms produce a fairly common parasite infection of man throughout the tropical world. They have a life cycle in various vertebrate hosts and one in the Phlebotomas fly. The latter requires about ten days for its completion, after which time, the fly's salivary secretions are infected with the Leptomonad forms of the parasite and when the fly feeds on a susceptible vertebrate, they probably are inoculated into the wound produced by the bite. Leishmania cause two types of infection in man, one, the visceral form, is confined to the endothelial cells of the capillaries of the internal organs, and the other, the cutaneous form, is primarily located in the endothelial cells of the capillaries and lymph spaces of the skin.

1. LEISHMANIA DONOVANI - LAVERAN AND MESNIL, 1903. This organism causes visceral Leishmaniasis, known clinically as Kala Azar or Dum Dum fever.

a. Morphology

In the vertebrate host L. donovani are small ovoid or round bodies with a vesicular nucleus and a kinetoplast, but never have a free flagellum. Their size varies from 1 M to 5 M in diameter. With Wright's or other polychrome stain of the Romanowsky type, the cytoplasm stains a pale blue, the nucleus appears as several bright red granules, the parabasal body stains deep purple, and the axoneme stains a faint pink. These tissue forms known as leishmania are practically always found within the cytoplasm of endothelial cells or macrophages. There may be as many as twenty of these parasites in one cell. In culture or in the invertebrate host, Phlebotomus papataci, the small leishmania forms elongate, develop a free flagellum and assume the leptomonad form. For a graphic representation of the morphology of this organism and of L. tropica, see figures 54 and 55.

b. Laboratory diagnosis.

See Laboratory Methods of the United States Army.

2. LEISHMANIA TROPICA - WRIGHT, 1903. Synonym L. braziliense. This organism causes cutaneous leishmaniasis, commonly known as Espundia, Oriental sore, Delhi boil or Bubas.

a. Morphology.

Its morphology is identical with that of L. donovani. In the cutaneous lesions it is found in the endothelial cells or macrophages which are present in great numbers in the dilated tissue spaces of the inflammatory lesion. The parasites may be quite scarce, but persistent search will usually reveal their presence in lesions typical for this disease.

b. Laboratory diagnosis.

See Laboratory Methods, Chapter V.

II. THE TRYPANOSOMES.

The trypanosomes are generally shaped like a sickle and are pointed at one or both ends. The convex margin has an undulating membrane, in the margin of which is imbedded the flagellum. The flagellum arises from a small granule, the blepharoplast, near the posterior extremity of the organism. It then passes along the free margin of the undulating membrane and may emerge at the anterior end of the organism as a free flagellum of variable length. Just posterior to the blepharoplast, there is usually a dark, shining, round or rod-like body known as the parabasal body. The nucleus is usually round or oval and centrally located. The cytoplasm is usually clear, but may contain granules of volutin which stain red with polychrome stains. Figure 56 shows diagrammatically a trypanosome and its internal structure.

The trypanosomes of medical importance are: (1) T. gambiense and T. rhodesiense, the causative organisms of African sleeping sickness; (2) T. cruzi, the causative organism of Chagas' disease or South American trypanosomiasis; (3) The confusing trypanosome, T. lewisi. This is a universal natural infection in rats, transmitted by the rat flea and is non-pathogenic for man. However, in the event rats are used as laboratory animals for inoculation with T. cruzi, T. lewisi, if already present in the rats' blood streams, may be confused with it.

1. TRYPANOSOMA GAMBIENSE - DUTTON, 1902.

This is the most common organism causing African sleeping sickness of man.

a. Morphology.

This trypanosome is characterized by marked polymorphism in the blood of mammals. It varies in length from 13 M to 40 M; long forms with a free flagellum and short forms with no free flagellum may be present in the same blood smear, plus all graduations between the two. The nucleus in T. gambiense is always centrally located.

b. Mode of transmission.

T. gambiense is transmitted to a new host through the invertebrate vector, the tsetse fly, the most important species being glossina palpalis. This transmission may be accomplished by: (1) Mechanically. If the tsetse fly feeds first on an infected individual and then almost immediately upon a non-infected individual, the fly may inoculate fresh infected blood from one to the other. (2) After cyclic development. After G. palpalis has fed upon an infected case of sleeping sickness, the trypanosomes taken in the blood feed, multiply and undergo a cyclic development in its mid-gut. In a few days they pass forward along the gut and eventually gain entrance to, and infect the salivary glands. They are now crithidial in form. In from twenty to thirty days, they develop into metacyclic trypanosomes and are now capable of being transmitted through the salivary secretions of the feeding fly to a new host. When once infected with T. gambiense, the tsetse fly remains so for life.

2. TRYPANOSOMA RHODESIENSE, - STEPHENS AND FANTHEM, 1910. This organism's morphology is identical with that of T. gambiense, except that in some of the short stumpy forms, without free flagella, the nucleus is situated in the posterior third of the body and may even be behind the parabasal body. Sleeping sickness due to T. rhodesiense is a much more severe infection than that due to T. gambiense.

3. TRYPANOSOMA CRUZI - CHAGAS, 1909. This is a fairly common trypanosome infecting man in tropical and subtropical South and Central America. It is an acute, fairly severe disease in children and is characterized by fever, thyroid, splenic and lymphatic glandular enlargement, followed by marked anemia and emaciation. In the adult, the infection usually runs a chronic course, with thyroid enlargement followed by symptoms of myxodema. The heart or any other organ may give rise to symptoms due to local invasion by this parasite.

a. Morphology and life cycle in man.

T. cruzi as seen in the peripheral blood is about 20 M in length. Its body tends to be shaped like the letter C. and the posterior end is sharply pointed like a wedge. The parabasal body is very conspicuous and oval in shape. There is a free flagellum. The nucleus is oval in shape and centrally located. There may be some variation in the width of the cytoplasmic body depending upon the age of the trypanosome; young forms are narrower than the old ones. After a variable period in the blood stream, the mature trypanosome invades muscle fibers or other tissue cells, rounds up, becomes leishmania in form and multiplies by binary fission. This multiplication of the leishmania forms continues until there may be as many as twenty or more parasites in a group. They then develop a short flagellum, the body lengthens, the parabasal body moves posteriorly and the young trypanosome now invade the blood stream. There may be considerable local tissue damage and destruction due to the invasion of tissue cells by this parasite.

b. Life cycle in the Reduvid.

Probably the most important vector is Conorhinus megistus. About six hours after the reduvid has fed upon an infected individual the ingested trypanosomes round up and assume leishmania forms. These forms rapidly multiply as they pass backwards along the intestinal tract. In the hind-gut they again develop flagella and in about twenty-five to thirty hours become crithidial forms. These forms multiply in the lumen of the pighian tubules. The crithidial forms now develop into metacyclic trypanosomes and when the reduvid again feeds, he deposits a small drop of fluid from his rectum upon the skin of the individual fed upon. This fluid excrement may contain great numbers of the trypanosomes which are capable of passing through mucous membranes or by means of small abrasions into the blood stream, thus starting the disease.

4. TRYPANOSOMA LEWISI - KENT, 1880.

This is the organism of rat trypanosomiasis. It is cosmopolitan in distribution and is not infectious for man. It is only of significance when it is confused with T. cruzi infections in laboratory animals. The distinguishing points morphologically are that the nucleus of T. lewisi is in the anterior third of the organism while that of T. cruzi is in the middle third, and the parabasal body in T. lewisi is rod-shaped while that of T. cruzi is oval. T. lewisi is transferred from rats to other laboratory animals with great difficulty, while T. cruzi transfers readily.

5. DIFFERENTIATION OF SPECIES

The main differential points are shown in figure 57.

6. LABORATORY DIAGNOSIS

This is made by demonstrating the trypanosomes in concentrated or unconcentrated stained smears, dark field preparations or wet mounts of blood. In some types of trypanosome infections smears from lymph gland aspirations are most apt to contain the organisms. When the trypanosomes are too scarce to be demonstrated by microscopic technique, culture or inoculation into laboratory animals should be resorted to. For technique in the above procedures see Laboratory Methods, Chapter V.

THE CILIATA

The members of this class have short, hair-like processes called cilia covering their bodies, which they use for locomotion and the captures of food. There is only one species in this class that is pathogenic for man, namely Balantidium coli.

I. BALANTIDIUM COLI - (MALMSTEN, 1857) STEIN, 1862

B. coli is a rare parasite of the human colon, and a common one of the colon of pigs. It is cosmopolitan in distribution. In man it is a true tissue parasite. It lives within the lumen of the large intestine primarily, but invades the mucosa and produces large sloughing ulcers, indistinguishable from those of Entamoeba histolytica. It acute cases it gives rise to an acute dysentery while vague abdominal discomfort is a common symptom in chronic cases.

1. MORPHOLOGY

a. TROPHOZOITES

This is the largest protozoon parasitizing man. It is oval in shape and its size varies from 60 to 220 M in its greatest diameter. At the anterior end is a depression called the peristome, which leads into the cytostome (mouth) and then the cytopharynx (pharynx). The body surface is covered with oblique, parallel rows of cilia. These cilia are greatest in number and length around the peristome. The cilia around the peristome beat rythmically toward the opening, thus creating currents which aid in the capture and ingestion of food. The endoplasm contains numerous food vacuoles and two contractile vacuoles, one near the caudal extremity, the other about midway in the body. Periodically in the live specimen these contractile vacuoles are seen to contract and expel their contents. At the rearmost point of the organism there is a small excretory opening, the cytopyge. Within the middle one-third of the endoplasm there is a large oval or kidney-shaped, dark staining body, the macronucleus, and near its concave side is a small typical protozoon nuclear structure with a large central karysome, the micronucleus. Reproduction is usually by transverse binary fission, although conjugation does occur in this species. Figure 58 shows the organism and its internal structure, and also its appearance in a lymph sinus of the intestinal mucosa.

b. CYSTS

The cysts of this parasite are more or less spherical in shape, measure 50 to 60 M in diameter and appear like a rounded trophozoite with a translucent shell around it. Each cyst contains a single organism and when excystation takes place only one daughter B. coli is produced.

c. TRANSMISSION

Transmission of this parasitic infection from infected to non-infected individuals only takes place when food or drink is contaminated by fecal excrement containing the cysts of B. coli. The cysts are only formed in the fecal currents of the colon. When living unencysted forms are passed in the feces, they die without undergoing encystation. If the living unencysted forms should be ingested, they would be killed by the acid gastric juices. Thus ingestion of viable cysts is the only natural way that this infection can be acquired.

2. LABORATORY DIAGNOSIS

In acute cases with a mucosanguineous diarrhea living trophozoites are found in feces. In chronic cases cysts are found in the feces. For technique see Laboratory Methods, Chapter V.

CHAPTER V

LABORATORY METHODS AND TECHNIQUE

GENERAL METHODS USED IN THE DIAGNOSIS OF PROTOZOA INFECTING THE BLOOD AND TISSUE

I. COLLECTION OF THE SPECIMEN

1. PRECAUTIONS

a. Time.

If it is possible, without detriment to the patient, select the most favorable time and method to find the suspected parasite in the clinical infection under consideration.

b. Use all available methods and repeated examinations.

If it is necessary, use all of the methods of examination available to establish a diagnosis, and do not be satisfied until repeated examinations at favorable times prove or disprove the clinical diagnosis.

c. Type of slides to use.

In making blood or tissue smears always use new slides or slides that are free from fogging or scratches.

d. General cleanliness of slides.

The slides should be clean and free from grease or good smears cannot be made.

e. Chemical cleanliness of equipment.

Slides and other laboratory equipment used in handling blood and tissue prior to and during staining should be chemically clean. If they have been cleaned in acid cleaning solutions, be sure that they are washed in sufficient tap water followed by distilled water so that their reaction is neutral.

2. UNSTAINED WET MOUNTS

The fresh unstained wet mounts are prepared by mixing one small drop of the patient's blood or aspirated tissue fluid with two drops of a 0.85% sodium chloride solution in distilled water on a slide, then covering

the material with a cover slip. There should not be so much fluid on the slide that the cover slip floats or it will be impossible to keep objects in the microscopic field. The cells in the preparation should not be so thick as to override one another and red cells should not form rouleaux. If they do so, the preparation should be discarded and a new one made using a greater dilution of the patient's blood.

3. THIN SMEARS

Thin smears for staining are prepared by securing blood or tissue juice from the patient, and then making thin smears on a clean slide as if for a differential white blood cell count. The smear should be so thin that the cells are in a single layer and do not override one another. When the smear is too thick, it is usually due to the use of too large a drop of the patient's blood or to the streaking slide being held at an angle of less than 35 degrees when the smear was made. The smear should not be blotted but allowed to rapidly air dry without the use of heat. Do not blow the breath upon the smear to hasten drying; it will lake the red blood cells. If smears are stained before they are dry, the cells will not properly fix, the stain will be precipitated and the result will be unsatisfactory.

4. THICK SMEARS.

Thick smears for staining are made by securing a large drop of the patient's blood on a clean glass slide, then spreading it by the use of a corner of another slide so that it covers an area about the size of a ten cent piece. The smear is then allowed to thoroughly air dry at room temperature or in 37°C. incubator. If the smear is not thoroughly dried before it is stained, the parasites will not stay on the slide.

5. COMBINED THICK AND THIN SMEARS.

The combined thick and thin smears for staining are prepared by making a thick smear on one end of the slide and a thin smear starting one-half inch from the thick smear, and then streaking it towards the opposite end of the slide. Draw a line with a wax pencil between the two smears and they are now ready for staining.

II. STAINS AND STAINING TECHNIQUE.

1. PRECAUTIONS

a. Quality of Stains.

Only good stains of proven quality should be used. Each lot of stain should be carefully tested on known slides before use on unknowns. In general, those that stain leucocytes and red blood cells satisfactorily will also stain blood and tissue protozoa.

b. Methyl Alcohol.

Methyl alcohol used in making up stains and in fixing smears should be chemically pure and free from acetone.

c. Distilled Water.

Distilled water used in diluting stains and in washing stained smears should be neutral in reaction or buffered to a pH of 6.8.

d. Staining Time.

The staining time for each step in any given method should be varied to give the best final result for any given lot of stain. The time quoted for various steps in each stain of this type is the average time found most satisfactory at the Army Medical School.

e. Heat.

After blood or tissue smears are stained, do not use heat to dry them, it will tend to fade the stain.

f. Stock Stain.

Great care should be exercised in seeing that no water gets into the stock stains prior to use in the staining technique, or the stain will be precipitated and rendered unfit for use.

2. WRIGHT'S STAIN AND STAINING TECHNIQUE.

The stain is the same as that given for blood work in "Laboratory Methods of the United States Army". The technique in staining for protozoa is as follows:

a. The Thin Smear.

(1) Flood the thoroughly dried blood smear with the stain for one-half to one and half minutes.

(2) Dilute the stain on the slide with an equal volume of neutral distilled water or enough of the water so that there is produced a metallic scum on top of the mixture. Allow this mixture to stand for three to five minutes.

(3) Thoroughly wash the stained blood film with neutral distilled water until it is light pink in color, then blot and allow to air dry.

b. Thick Smears.

Thick smears are best stained by the Giemsa method. However, if this stain is not available, they may be stained with Wright's stain, using the following method:

(1) Immerse for 10 minutes the thoroughly dried thick smear in a solution made of:

Formalin	5 cc.
Acetic acid	1 cc.
Distilled water q.s.ad	100 cc.

This solution fixes the parasites and white blood cells, but dissolves the red cells.

(2) Remove the smear and wash thoroughly in tap water following with distilled water.

(3) Allow slide to thoroughly dry and then stain by Wright's method as for a thin smear.

c. Combined Thick and Thin Smears.

Proceed as for a thick smear, but, be careful to immerse only the thick smear in the acidulated formaldehyde solution. If the thin smear comes in contact with this solution, the red cells will be dissolved out and the smear will be useless.

Failure to stain by Wright's method is usually due to insufficient lapse of time after diluting the stain with distilled water, or to contamination of the stain, or other reagents, or material, with acid. The precipitation of granules of stain on the blood film is either due to improper drying of blood films before starting the stain, introduction of water into the stock stain, or too much evaporation of the alcoholic stain before dilution. Red cells stained blue, except for the occasional cells showing polychromatophilia, are either over-stained, (too much time allowed after diluting the stain) or have been insufficiently washed during the last stage of the staining process.

3. GIEMSA STAIN.

This stain is as good as that of Wright and in addition is more permanent. The good commercial stains are usually much more satisfactory than those made up in small amount in a laboratory. If it is necessary to make up this stain, the formula is as follows:

Azur II eosin	0.3 grams
Azur II	0.8 grams
Glycerol, C. P., (Sp. gr. 1.26 and water content not over 1.5%)	25 cc.
Methyl alcohol, C. P., acetone free	25 cc.

Dissolve the Azur II and Azur II eosin in the glycerol which has previously been heated to 60°C. Now add the methyl alcohol and allow the mixture to stand for 12 hours, then filter and the stain is ready for use.

The actual staining solution is made by the proportionate mixing of one drop of the above stock solution with one cubic centimeter of neutral distilled water. Distilled water containing 1 cc. of 0.5% sodium carbonate in each 100 cc. is probably the best diluent. Seventy-five cubic centimeters of the diluted stain will stain about twenty-five slides. After the stain is diluted, it should be used as soon as possible as it deteriorates and will not stain well after twelve hours. Staining technique by the Giemsa method is as follows:

a. The Thin Smear.

The thin smear must be fixed by immersion in methyl alcohol for five minutes. It is then removed, dried and placed in the diluted Giemsa stain for thirty minutes if the stain is for malaria and forty-five to sixty minutes if it is for flagellates. Remove from the staining solution, rinse with distilled water until the blood film is light pink in color, then stand it on end and allow to drain dry. When thoroughly dry, the smear is ready to be viewed.

b. The Thick Smear.

The thick smear is stained in exactly the same way as the thin smear, except that it is not fixed in methyl alcohol prior to immersion in the staining solution.

c. The Combined Thick and Thin Smear.

The combined thick and thin smear is stained exactly in the same way as the thin smear except that great care must be taken to see that the methyl alcohol does not get on the thick smear. If it should do so, the red cells of the thick smear will not lake out and the thick blood smear will be useless for diagnostic purposes.

4. PROPER STAINING.

The criteria for a good blood or tissue stain when used to detect the presence of protozoan parasites are:

a. The red cells should be pink to a copper color.

b. The white blood cells should show clear cut nuclear staining with good contrast between the nucleus, cytoplasm and cytoplasmic granules.

c. The parasites should show light blue stained cytoplasm with bright red or violet chromatin and their morphological structure should be clearly defined.

SPECIAL LABORATORY METHODS USED IN THE DIAGNOSIS of protozoa infecting the blood and tissue.

I. MALARIA.

1. COLLECTION OF THE SPECIMEN.

a. Time.

The most favorable time to find malarial parasites in the blood stream in a clinical case of malaria is the period starting twelve hours after one chill up to one hour before the next chill.

b. Effect of Quinine Therapy.

Quinine therapy within four days prior to the taking of a sample of blood to be examined for malaria will usually make it very difficult or impossible to demonstrate the parasites in the peripheral blood, except by the thick smear method.

2. FRESH UNSTAINED WET MOUNTS.

The fresh unstained wet mounts are prepared as outlined under general methods. Except for plasmodium vivax, the recognition of malarial parasites in these mounts is quite difficult and requires experience. The pigment in the clear hyaline body of the amoeboid forms of P. vivax moves quite rapidly and can be easily seen under the oil immersion objective, but P. malariae and P. falciparum parasites are less readily seen. For the above reason, this method of diagnosing malaria is generally unsatisfactory and should not be used except when stains are not available.

3. THIN STAINED SMEARS.

The preparation of thin stained smears as outlined under general methods, stained with Wright's or Giemsa stain, is the method of choice in the routine diagnosis of malaria.

4. THICK STAINED SMEARS AND COMBINED THICK AND THIN STAINED SMEARS.

The thick stained smear and the combined thick and thin stained smears are prepared and stained by Giemsa or Wright's method as outlined under general methods. They are the methods of choice in searching for malarial parasites in carriers, clinical cases that have very few parasites in the peripheral blood and in malarial surveys. The appearance of the parasites in a stained thick preparation after the red cells have been laked out is quite different from that in stained thin preparations. They are quite typical, but the body of the parasite may be distorted in shape due to the destruction of the red blood cells. Therefore, where possible, the novice should not use this method until he or she has become thoroughly familiar with the malarial parasites and those confusing objects that are found in the thin stained smear. A convenient way of handling and staining large numbers of thick smears in a malarial survey is the one outlined by Barber and Kemp of the U. S. Public Health Service. "In handling large numbers of thick smears it is convenient to carry out the technique in groups of 25 slides. With this in mind, the thick film is placed about one inch from one end of the slide and the other end is used for labeling. The slides are assembled in groups, a cardboard 1/16 to 1/8 inch thick and $1\frac{1}{2}$ inches long, is inserted between the slides at the labelled ends and the whole fastened together by means of a stout rubber band. The entire block may now be stained and dried as a single unit. Figure 59 illustrates such a block.

5. CULTURAL METHODS

The cultivation of the plasmodia of malaria by the methods now available is of no practical value in the diagnosis of this infection. Its chief use at present is to demonstrate, for teaching purposes, the forms of P. falciparum that are usually only found in the capillaries of the internal organs. If the technique of malarial culture is desired it may be found in the Journal of Experimental Medicine, XVI, 567, 1912.

6. INOCULATION OF LABORATORY ANIMALS.

There are no laboratory animals that are susceptible to human malaria.

7. OBJECTS THAT MAY BE CONFUSED WITH MALARIA.

Objects that may be confused with malaria in the stained blood smears when superimposed on a red cell are: Blood platelets and precipitated stain. Sometimes abnormal red cells showing young nucleated forms or basophilic degeneration may also lead to confusion. Malarial parasites have a definite body shape, internal arrangement and characteristic staining properties which, when once familiar with them, should lead to easy differentiation from other objects.

8. LABORATORY DIAGNOSIS.

The laboratory diagnosis of malaria infection in man consists of finding and recognizing the malarial parasites, and their type, when present in the blood preparations made from suspected malarial fever patients. In examining preparations made for malaria the oil immersion objective should be used. Malarial parasites are best seen when the light coming through the substage is slightly reduced. The proper amount of light may be obtained by moving the slide until a blood platelet is centered in the field, then adjust the substage so that the maximum definition of its morphological detail is obtained. In searching for the parasites the slide should be covered in an orderly manner moving back and forth over the smear so as not to repeat any field previously examined. Never make a diagnosis on the first parasite found, cover enough of the slide so that if two species of malarial parasites are present you will find them. If in doubt about any single abnormal parasite found, remember that where there is one malarial parasite there are bound to be more and careful search will usually reveal an easily recognizable form. Early diagnosis and treatment is of utmost importance if a favorable prognosis is to be expected in P. falciparum infections, owing to its tendency to produce early and unexpected cerebral complications. Therefore, if P. falciparum infections are found in the area in which you are working and you have a blood smear in which you are certain there are malarial parasites, but you are unable to determine the type, report the case as positive for malaria so that treatment may be started. Then make additional smears and carefully study to determine the type.

II. TRYPANOSOMES

1. COLLECTION OF THE SPECIMEN.

a. Time

The most favorable time to find these organisms in a suspected infection is during periods of pyrexia.

b. Blood.

In most cases of trypanosomiasis there are usually very few organisms present in the blood stream; therefore, it is necessary to use concentration methods as well as direct preparations in searching for those parasites. Such a concentration method is as follows:

A sterile venipuncture is done and 10 cc. of blood are withdrawn and immediately mixed with 40 cc. of sterile warm (37° C.) distilled water in a 50 cc. sterile centrifuge tube. The tube is agitated and as soon as hemolysis is complete it is centrifuged at 2500 revolutions per minute, or over, for 5 minutes. Then decant the supernatant fluid and oil the trypanosomes present in the sample of blood will be found in the residue which can then be examined microscopically or if it has been kept sterile, used to inoculate culture media.

c. Lymph Gland Puncture.

This method is the one most apt to reveal positive results in the diagnosis of trypanosomiasis. Puncture one of the enlarged lymph glands under aseptic technique with a Luer syringe equipped with a 19 gauge needle and aspirate some of the gland substance. The aspirated gland material may then be examined by microscopic, cultural or animal inoculation methods.

d. Spinal Fluid Examination.

Spinal fluid examination is probably only of value in African sleeping sickness. Due to the small number of trypanosomes present in the spinal fluid it is usually necessary to centrifuge the specimen and then examine the residue so obtained to demonstrate their presence.

e. Tissue.

T. cruzi is the only trypanosome that forms a nidus and propagates in tissue. The pathological examination of biopsy material from localized swellings or lymph glands in suspected cases of this disease may reveal its presence. In autopsy material, heart, thyroid tissue, brain and bone marrow are most apt to harbor this parasite.

2. SLIDE PREPARATIONS.

Fresh unstained wet mounts, thin stained smears and thick

stained smears are prepared as outlined under general methods.

3. CULTURE IN LABORATORY MEDIA.

Concentrated laked blood, aspirated contents of lymph glands, spinal fluid or biopsy tissue from cases of trypanosomiasis when inoculated into suitable media may yield cultures positive for trypanosomes. The various species arecultured as follows:

a. T. Cruzi.

(1) One of the best media upon which to culture T. cruzi is that originated by Novy, MacNeal, and Nicolle (N. M. N. medium) and is made as follows:

Agar	14 Grams.
Sodium chloride	6 grams.
Distilled water	900 cc.

Mix and dissolve by means of heat, then tube in 6 cc. amounts and autoclave for 30 minutes under 20 pounds steam pressure. Remove the sterilized tubed nedia and allow it to cool to 48° C. Then under aseptic conditions add 2 cc. of sterile defibrinated rabbit's blood to each tube, mix well and slant. Slanted tubes should be placed in the ice box to cool and harden so that they will have the maximum amount of water of condensation. When cool the cotton plugs in each tube should be covered with a rubber cap to prevent evaporation of the water from the media. The tubes should be tested for sterility by incubation at 37°C. for 24 hours before they are used. Trypanosomes will not grow in the presence of bacterial contamination.

(2) Inoculate the suspected material into the water of condensation of N. M. N. medium and incubate at 25°C., examining every 48 hours for 10 days. Leishmania forms, crithidial and trypanosome forms ordinarily found in the reduvid will appear in the culture. Subculture, however, is quite difficult.

b. T. Gambiense and T. Rhodesiense.

T. gambiense and T. rhodesiense are difficult to culture but may grow on the above N. M. N. medium if 1% glucose is added and the culture is incubated at 32°C. The forms that develop in the culture will be the crithidial and metacyclic forms ordinarily found in the tsetse fly.

4. INOCULATION OF LABORATORY ANIMALS.

Inoculation of laboratory animals is used to establish a diagnosis when other methods fail. Great care should be used in selecting the proper susceptible animal as given in the differential chart on trypanosomes and ruling out the possibility of a natural infection existing at the time

of inoculation. Material obtained as outlined under the collection of specimens is inoculated intraperitoneally into the animal selected.

5. LABORATORY DIAGNOSIS

The laboratory diagnosis is arrived at by studying the material gathered according to the above outline and correlating it with the differential table plus life histories of the organisms. The presence of autoagglutination in a patient's blood is suggestive of trypanosomiasis.

III. LEISHMANIASIS.

1. COLLECTION OF THE SPECIMEN.

a. Blood.

Leishmania are rarely found in the circulating blood and when present they are found in the macrophages and polymorphonuclear leucocytes.

b. Liver Puncture.

When visceral leishmaniasis can not be diagnosed by blood examinations, this is the method of choice, and of all methods it is most likely to yield positive results. This procedure is not without danger, however, but can usually be done safely as follows: The excursions of the diaphragm are limited by putting on a tight abdominal binder. Then, cautioning the patient not to move, under a local skin anesthetic, insert a 2 inch, 16 gauge Luer needle attached to a 10 cc. Luer syringe containing 2 cc. of saline, through the skin just above the 8th rib in the right midaxillary line. Keep the needle and syringe at right angles to the skin and after the needle has penetrated 1 inch inject the 2 cc. of saline and then insert the needle to its full length. Then aspirate a small amount of material and withdrew the syringe. The aspirated contents may then be used to make smears for staining or for inoculation of culture media.

c. Spleen Puncture.

Securing material for the diagnosis of visceral leishmaniasis in this manner is attended with great danger of hemorrhage. However if necessary to make the diagnosis through this procedure, it is performed in much the same manner as liver puncture.

d. Skin Lesions.

Material for diagnosing infection with L. tropica is obtained by scraping the edge of the cutaneous ulcers or using a Luer syringe with a 19 gauge needle to aspirate some of the contents of a skin nodule. This material is then made into thin smears for staining. Material from skin lesions is usually too heavily contaminated by bacteria to be successfully cultured.

2. CULTURE ON LABORATORY MEDIA.

Culture on laboratory media is carried out by inoculating material obtained by the above procedures into the water of condensation of a slant of N. M. N. medium which is then incubated at 25° C. The material inoculated must be free from bacterial contamination or leishmania will not grow in the culture. Within 3 to 10 days in successful culture, the water of condensation will contain numerous rosettes of leptomonad forms of the leishmania which may be demonstrated by wetfresh mounts of stained preparations.

3. INOCULATION OF LABORATORY ANIMALS.

Localized cutaneous infections can sometimes be produced by inoculating material infected with leishmania into the skin of dogs, cats, rats, mice, guinea pigs, or monkeys. The hamster, however, is the animal of choice and infection with leishmania can be produced readily in it.

4. THE ALDEHYDE TEST.

This test is performed by adding two drops of commercial (37%) formalin to 1 cc. of serum from the suspected case. If the infection is due to L. donovani, the serum will jell and look like the cooked white of an egg. This test, however, is not specific, for it may give a positive result in some cases of tuberculosis, leprosy or malaria.

5. LABORATORY DIAGNOSIS.

The laboratory diagnosis of L. donovani or L. tropica i made by finding these organisms in the leucocyted and endothelial cells stained smears, in cultures or in the lesions of inoculated laboratory animals.

GENERAL METHODS USED IN THE DIAGNOSIS OF THE PROTOZOAN PARASITES AND SAPROPHYTES OF THE MUCOUS SURFACES OF MAN

I. COLLECTION OF THE SPECIMEN.

1. PRECAUTIONS.

a. Collecting the Specimen

The specimen should be directly collected in clean covered receptacles (bed pans, swabs in test tubes containing ½ cc. of physiological saline, syringes, bottles or dropper), preferably sterilized by heat. These receptacles should not be sterilized by chemical disinfectants as protozoa in the vegetative stage are easily killed and quickly undergo autolysis in the presence of only small amounts of such chemicals. If the receptacles are not properly cleaned and sterilized there is always the possibility of introducing free-living protozoa into the specimen and thus confusing the picture

b. Handling the Specimen Prior to Examination.

(1) Specimens for Examination Locally.

These specimens should be kept in the original receptacle used for collection until examined. The material should be kept at a temperature of 37°C. as all vegetative organisms in this group are quite sensitive to chilling and are also rapidly killed by temperatures of 45°C. or higher and by drying. Therefore, when delivered for diagnosis, the specimen should have its original moisture content or examination for these organisms will be useless.

(2) Specimens for Shipment.

(a) Vegetative organisms will not stand routine shipment without previous fixation. Cysts, found in feces, are the only forms that can be satisfactorily shipped in the fresh state. The cysts should be concentrated and washed with tap water until quite free from fecal material as they rapidly die in the feces as passed, but will live from twenty to two hundred and fifty days, depending on the temperature, in the washed state.

(b) Concentration and Fixation of Mucous Surface Protozoal Specimens Containing Trophozoites Preparatory to Shipment.

-1- Fecal specimens. Thoroughly emulsify 20 cc. of feces in 200 cc. of warm (37° C.) physiological saline in a settling flask or tall narrow cylinder, allow to stand for five minutes and then decant the supernatant fluid into two 50 cc. centrifuge tubes. Centrifuge the material at 1850 RPM for five minutes, then decant off the supernatant fluid, save the precipitated residue of one tube for fresh examination and to the tube containing the other precipitate add 25 cc. of fresh Schaudinn's fixing

solution. Thoroughly mix the precipitate and the fixative and allow the mixture to stand for at least one hour, preferably 24 hours.

-2- Cultures and other liquid specimens. Pipette the fluid containing the organisms directly into a 50 cc. centrifuge tube and then centrifuge and fix the material as indicated above.

-3- After the one specimen has been fixed by Schaudinn's solution the required length of time, the solution should be removed from the specimen by first diluting it with an equal volume of distilled water, then centrifuge the mixture so obtained at 1850 RPM for 5 minutes and decant off the supernatant fluid. Now add 50 cc. of 95% ethyl alcohol to the precipitated residue and it will then be ready for shipment in a suitable container. Ship the fixed and unfixed specimens obtained as above to the corps area or other central laboratory for diagnosis.

c. Time of Examining the Specimen After Collection.

All specimens should be examined at the earliest possible opportunity after collection. Those types of protozoa rapidly degenerate and the possibility of a positive diagnosis is inversely proportional to the elapsed time after collection of the specimen.

d. The Necessity for Multiple Specimens and Examination.

In patients infected with protozoa of this type, the number of organisms present on an infected surface at any given time depends upon the type of pathology produced, the bacterial flora present and the resistance of the host. Because of this multiplicity of governing factors, protozoa usually appear in showers; they may be present in great numbers one day and relatively scarce the next. Therefore, to arrive at anything like a satisfactory diagnosis in questionable cases, repeated examinations, possibly under different conditions, should be made on consecutive days.

2. SPECIMENS FROM THE BUCCAL CAVITY AND THE GENITO-URINARY TRACT.

These are best collected directly from the lesions by using swabs moistened with physiological saline, or by aspirating some of the mucous secretions by means of a syringe or dropper. This material is then immediatly examined microscopically or by culture.

3. SPECIMENS FROM THE GASTRO-INTESTINAL TRACT.

a. Precautions.

(1) It is practically impossible to find protozoa in a stool after an oil cathartic, e.g. castor oil, mineral oil, or following a barium meal or enema. Therefore, in the event any of the above has been used examination for protozoa in the feces should be delayed for at least 72 hours

(2) The portions of a stool most likely to contain parasitic protozoa are those showing blood or mucus. In formed stools, small flecks of mucus or mucus and blood can always be found on the surface of

the specimen. In semiformed or liquid stools if the specimen is examined carefully, mucus and blood can also be found.

(3) Specimens collected by means of an enema are usually unsatisfactory.

b. How and When to Collect the Specimen.

Feces should be passed directly into a thoroughly clean, warmed bed pan and immediately taken to the laboratory for examination. The best time to secure a specimen is at about 9 A.M. At this time the patient, due to the stimulation of peristalsis by breakfast, will be able to cooperate best and the laboratory personnel should be able to give the specimen immediate attention.

c. Stage of Organisms Found in Different Types of Stools.

Formed stools usually contain cysts or precystic protozoa only. Semiformed and liquid stools will ordinarily contain only vegetative forms and it is these types of stools that best afford an opportunity to diagnose a protozoan infection. If the patient is not already passing such a stool, and it is necessary to rule out the possibility of a parasitic protozoan infection, give a saline cathartic and collect the second or third liquid stool passed for examination.

d. Concentration Methods.

This method is only applicable to cysts for which it is the procedure of choice. For all practical purposes only formed or semiformed stools will contain cysts and if they can not be demonstrated in the unconcentrated specimen the following procedure should be used. Select a portion of feces about the size of a walnut and thoroughly emulsify it by means of a tongue depressor in 200 cc. of distilled water contained in a graduate or settling flask. Allow this material to stand for 30 minutes to give the heavier particles time to settle out; now decant the supernatant fluid into a liter graduate or flask and add enough distilled water to fill the container. The diluted specimen is then placed in the refrigerator overnight to allow the cysts to settle to the bottom. The following morning decant or siphon off the supernatant fluid and discard it. The residue is then examined directly for cysts or it may be washed one or more times and further concentrated by the following method. Transfer the residue to a 50 cc. centrifuge tube and dilute with enough distilled water to fill the tube, thoroughly mix and then centrifuge the specimen for two minutes at 2000 RPM. The supernatant fluid is then decanted and cysts if present will be found in the precipitated residue.

e. Proctoscopic Examination.

If the pathological lesions are in the rectum or sigmoid specimens may be obtained by means of the protoscope or sigmoidoscope and examined immediately. These are more apt to yield protozoa than the passed feces. However, because of the attending discomfort and perhaps pain to

the patient, this method should be used only after it has been demonstrated that the feces are negative.

II. PREPARATION OF THE SPECIMEN FOR MICROSCOPIC EXAMINATION.

1. THE WET FRESH UNSTAINED SPECIMEN.

a. Feces and Mucus Specimens.

Warm a clean slide so that it feels comfortable when touched to the back of the hand. Then secure a small piece of mucus or mucus and blood from the fresh specimen by means of a wire loop or wooden applicator and thoroughly emulsify it in one drop of physiological saline on the middle of the slide. Now take a clean number one cover slip between the thumb and forefinger of the right hand, contact the slide with one edge of the cover slip near the drop but not touching it, push the slip along the surface of the slide until its edge contacts the drop, rock it slightly from side to side to allow a portion of the fluid to come under the edge of the slip, then let the cover slip drop from between the fingers allowing it to fall on the side. The fluid portion of the drop on the slide will then automatically be drawn by capillary attraction under the slip, while the solid particles will be excluded. This method insures a thin even preparation of not too great a density (when newsprint is viewed through it the words are legible) and insures even apposition of the cover slip to the slide. The preparation is now ready for examination, but if it is to be kept on a worm stage for any period of time it should be ringed with vaseline.

b. Culture and Concentrated Cyst Specimens.

Culture and concentrated cyst specimens are prepared for microscopic examination exactly as above except that because of their fluid nature they are not mixed with physiological saline prior to applying the cover slip.

2. THE STAINED PREPARATION (See technique under fixation and staining).

III. FIXATION AND FIXING TECHNIQUE.

1. PRECAUTIONS.

a. Type of Fixatives.

A fixative should be selected that has approximately the same surface tension as the material to be fixed, or it will be difficult to keep the organisms on the slide, e.g. protozoa should not be fixed in solutions that have more than 40% alcoholic content.

b. Time Needed for Fixation.

Always allow the specimen to remain in the fixative for

at least the minimum time quoted. It is possible to secure some fixation in less time, but the increase in the quality of stains produced after adequate fixation more than justifies the additional time allotted to this step.

c. Condition of Fixative.

The fixative should always befresh and the number of slide preparations fixed in a given amount limited, i. e., seventy-five cubic centimeters of fixative will only fix satisfactorily about twenty-five slide preparations.

d. Condition of Specimen to be Fixed.

The specimen to be fixed should be fresh and the organisms should not show any evidence of degeneration. If the specimen is old and the organisms are degenerated, good fixation and staining will be impossible.

2. FIXATIVES.

a. Schaudinn's Solution:

Mercuric chloride, $HgCl_2$, C.P., saturated solution in physiological saline	65 cc.
Ethyl alcohol, 95%	35 cc.
Acetic acid, glacial, C.P.	5 cc.

The glacial acetic acid and the ethyl alcohol should be added to the bichloride of mercury solution just before use.

b. Formol Sublimate Solution:

Mercuric chloride, saturated solution in physiological saline	90 cc.
Formaldehyde (37%)	10 cc.

3. FIXATION TECHNIQUE.

a. Flagellates, ciliates and cysts.

If the material to be fixed is not in the liquid state, mix enough physiological saline with it to form a thin watery solution. Now rub well into one half of the surface of a clean slide one small drop of fresh egg white or normal horse serum, then put one large drop of the specimen on this surface and spread it evenly. Allow the preparation to dry until the fluid portion of the specimen will no longer run when the slide is tilted, then drop the slide, film slide down, into 50 cc. of one of the above fixing solutions contained in one half of a Petri dish. After ten minutes turn the slide over or place it in a Coplin jar three-fourths full of the same fixative and allow it to remain for one to two hours.

b. Amoeba Trophozoites.

Slide preparations for fixing and staining these organisms are made in the same way as under (a) except that as soon as the drop of material is placed on the slide a cover slip is dropped on it. The preparation is then allowed to set for three minutes and is then placed in a Coplin jar. The fixative is then carefully added and is allowed to act for 2 hours. After fixation the cover slips are removed from each specimen and the slides are then ready to be carried through the routine of staining. This method of putting the cover slip on the preparation makes it possible to inspect the slide for amoebae prior to fixation and in addition is a great help in keeping the amoebae on the slide during fixation.

c. Preserving Specimens after Fixation.

The preparations after fixation should be rinsed in tap water and then placed in 70% ethyl alcohol; they can either be preserved for future staining in 95% ethyl alcohol, or stained immediately.

IV. STAINS AND STAINING TECHNIQUE.

1. PRECAUTIONS.

a. Stains.

A good quality of hematoxylin should be procured and when made up it should be thoroughly ripened before use. Test the stains on a known specimen before using them on an unknown.

b. Age of Material after Fixation.

Specimens should be stained as soon as practicable after fixation because fixed specimens gradually lose their ability to take the stain.

c. Drying of the Specimen.

The specimen should never be allowed to dry at any stage in the technique as it causes the organisms to shrink and become distorted in shape.

d. Washing after Destaining.

If the destaining agent is not thoroughly washed out of the specimen it will fade the stain.

c. Dehydration of the Specimen for Permanent Stains.

If the specimen is not properly dehydrated before clearing in xylol, the xylol will become milky and the slide when viewed microscopically after mounting in balsam will appear blurred.

2. THE IODINE STAIN.

This is a temporary stain used for quick diagnosis only and it must be made up fresh every 10 days or it will not stain properly.

a. Formula:

Iodine	0.05 gram
Potassium Iodide	0.10 "
Distilled water	100 cc.
Glacial acetic acid	1.0 "

b. Technique for Trophozoites.

This method is principally of value in studying amebae. After findingtrophozoites and studying them in the fresh wet unstained mount the nuclear details can be clearly visualized and studied microscopically if the cover slip is raised at one edge and a drop of the iodine stain is thoroughly mixed with the contents under the slip.

c. Technique for Cysts.

Mix on a clean slide one drop of the iodine stain with one drop of the material containing cysts, apply the cover slip as explained under fresh wet unstained mounts and then examine microscopically.

3. HEMATOXYLIN STAINS.

a. Heidenhain's Iron Hematoxylin.

This is the best stain for protozoa.

Hematoxylin	1 gram
Ethyl Alcohol, 95%	10 cc.
Distilled water	90 cc.
Thymol	1 crystal

Dissolve the hematoxylin in the alcohol, add the distilled water and thymol, then allow to ripen in a clear glass-stoppered bottle exposed to the sun for one month.

b. Harris' Hematoxylin.

This is a good stain and is the one most readily prepared.

Hematoxylin	1 gram
Ethyl alcohol, 95%	10 cc.

Dissolve the hematoxylin in the alcohol.

Alum (ammonium or potassium)	20 grams
Distilled water	200 cc.

Dissolve the alum in the water by the aid of heat, and add the hematoxylin solution. Bring the misture to a boil as rapidly as possible, and then add one-half gram of yellow oxide of mercury. The solution

at once assumes a dark purple color. As soon as this occurs, remove the vessel containing the solution from the flame and cool rapidly by plunging into a basin of ice water. As soon as the solution is cool it is ready for use. The addition to the stain of glacial acetic acid to a concentration of 4 per cent is supposed to increase the precision of the nuclear staining.

c. Mayer's Hemalum.

This is a fairly good and reliable stain.

Hematoxylin	1 gram
Distilled water	1000 cc.
Sodium iodate, C.P.	0.2 gm.
Potassium alum, C.P.	50 Grams

Dissolve the hematoxylin in the distilled water, add the sodium iodate and the potassium alum, then allow to ripen in the sun for one month in a clear glass-stoppered bottle. Filter the stain before using.

4. HEMATOXYLIN STAINING TECHNIQUE.

a. Slide Method.

The fixed and washed slide specimens prepared as outlined previously are carried through the successive steps in staining as follows:

(1) Wash 10 minutes in 70% ethyl alcohol.
(2) Wash 10 minutes in 70% ethyl alcohol to which has been added sufficient iodine stain to produce a light mahogany color.
(3) Wash 10 minutes in 70% ethyl alcohol.
(4) Mordant in the following solution(24 hours if using Heidenhain's stain, 1 hour if using Harris' or Mayer's stain).

Iron alum (ferric ammonium sulphate), 4% aqueous solution	1 part
Ethyl Alcohol, 50%	10 parts

(5) Wash for 5 minutes in 70% ethyl alcohol.
(6) Stain for 1 hour if using Harris' or Mayer's hemalum and for 24 hours if using Heidenhain's iron hematoxylin.
(7) Remove from stain and wash in two changes of tap water.
(8) Destain in the following:

Iron alum, 2 to 4% aqueous solution	1 part
Ethyl alcohol, 50%	10 parts

Differentiate by agitating each slide separately in the above solution until a light grey to blue tinge predominates; control the exact point by observing the staining definition of the organism under the microscope every few minutes.
(9) Rinse in tap water, then wash for 10 minutes each in three separate dishes of 70% ethyl alcohol.
(10) Begin dehydration by two changes of 95% ethyl alcohol for 5 minutes each.

(11) Complete dehydration by two changes of absolute ethyl alcohol for 10 minutes each.

(12) Replace the absolute alcohol in the specimen by two changes of xylol for 10 minutes each.

(13) Mount in Canada balsam.

b. Method of Staining Protozoa in Bulk.

In the staining of mucous surface protozoa of man there are almost as many variations in staining technique as there are workers in the field. The chief difficulties encountered in all of the better techniques of staining are: (1) keeping the organisms on the slide or cover slip during fixation and staining, (2) keeping the organisms free from distortion and clear from the debris so that their internal structures are not obscured, (3) carrying out proper differentiation of the internal structures of the organisms at the time of destaining, (4) having a sufficient number of well stained organisms on the slide after staining so that a diagnosis will not have to be made on a few more or less typical organisms.

The following staining method not only satisfies the above requirements, but also affords a method of concentration, thereby making it possible to secure satisfactory stains, from specimens containing very few organisms, that could not be stained by other methods. The method of staining differs from other standard hematoxylin staining methods only in that the organisms are not fixed and stained on slides or cover slips, but are fixed and stained in bulk. The organisms are concentrated and then carried through the steps of fixation and staining in 50 cc. centrifuge tubes. Very few of the organisms present in the original specimen are lost during fixation, staining and mounting. The organisms are natural and life like and are not distorted by the reagents or manipulations used.

(1) Precautions.

(a) There should be no delay in concentration, examination and fixation of trophozoites after they have been secured from the patient. Needless to say degenerated organisms will not take a satisfactory stain. It is not necessary to be so cautious in the immediate fixation of cysts, but as a rule the quality of protozoan staining possible to secure, is inversely proportionate to the elapsed time after securing the organisms from the host or culture, and their subsequent fixation.

(b) Destaining must be checked by frequent microscopic observations of the organisms being differentiated. Care should be exercised to carry the destaining to the point that there is sharp differential detail between the nuclear structures and the cytoplasm. The common tendency with this technique is not to carry the destaining far enough. The organisms should be checked for structural detail after the

acid destain has been neutralized and if there has been insufficient destaining they should be carried back into acid alcohol and further destained until the desired degree of differentiation has been secured.

(c) The stained material must be properly dehydrated before clearing in xylol.

(d) Discard the supernatant fluid decanted off after centrifuging, in each step of the procedure, or if the alcohols and stains are to be used over again, filter through a Berkefield N filter as there is danger of carrying organisms in used alcohols and stains to the next case subsequently stained.

(e) The material being stained may be left for 24 to 48 hours additional time in any step of the staining procedure with the exception of the fixing solution and the acid alcohol destaining reagent. This allows the staining procedure to be carried out without interfering with the routine work in a laboratory.

(2) Concentration and Fixation.

(a) Fecal specimens. Thoroughly emulsify 20 cc. of feces in 200 cc. of warm (37°C.) physiological saline in a settling flask or tall narrow cylinder, allow to stand for five minutes and then decant the supernatant fluid into two 50 cc. centrifuge tubes. Centrifuge the material at 1850 RPM for five minutes, then decant off the supernatant fluid, save the precipitated residue of one tube for fresh examination and to the tube containing the other precipitate add 25 cc. of fresh Schaudinn's fixing solution. Thoroughly mix the precipitate and the fixative and allow the mixture to stand for at least one hour, preferably 24 hours.

(b) Cultures and other liquid specimens. Pipette the fluid containing the organisms directly into a 50 cc. centrifuge tube and then centrifuge and fix the material as indicated above.

(3) Staining.

(Between each step in the subsequent procedure the material is centrifuged at 1850 RPM for 5 minutes, then the supernatant fluid is decanted off and the next solution added to the precipitated residue which is then thoroughly emulsified by rotation or stirring.)

(a) Wash the fixed material two times with distilled water.

(b) Wash 10 minutes with 70% ethyl alcohol (containing enough Gram's iodine to give it a light brown color.)

(c) Wash 10 minutes with 70% ethyl alcohol.

(d) Stain by adding Harris's or Delafield's hematoxylin for from 1 to 24 hours.

(e) Wash with tap water.

(f) Destain by adding about 20 cc. of acid alcohol (1% HCl in 70% ethyl alcohol) to the stained precipitate in the centrifuge tube. Mix the precipitate and the destaining solution and occasionally stir the mixture with a wooden applicator stick. From time to time check the progress of destaining by taking one drop of the mixture, placing it on a slide, applying a cover slip and then observing the progress of nuclear differentiation of the organisms under the high dry power of the microscope. The organisms will be fairly easy to find in the average case and destaining should be allowed to go on until the cytoplasm is practically colorless and the nucleus stands out sharp and clear. As soon as the required definition has been obtained, add sufficient ammonia water (5 drops NH_4OH in 50 cc. of distilled water) to neutralize the acid alcohol and turn the solution bright blue.

(g) Wash with tap water.

(h) Dehydrate with: 70% ethyl alcohol 10 minutes.
95% " " " "
95% " " " "
absolute " " " "
absolute " " " "

(i) Clear in xylol.

(j) Thoroughly emulsify the stained cleared mixture in a minimum of xylol, breaking up all small clumps, then add sufficient thick Canada balsam to make a syrupy mixture. Then without further centrifugation place one drop of the balsam containing a suspension of the stained organisms on a clean slide, apply a cover slip and allow to dry.

Permanent class room sets and multiple permanent mounts for file and consultation can be easily prepared by making more than one preparation in the final step. Stained material suspended in balsam can be kept in bottles and mounted on slides whenever desired.

V. CULTURAL METHODS

1. MEDIA

a. The Boeck-Drbohlav Medium (R.E.S. Medium).

The Boeck-Drbohlav medium is the one most generally used for the protozoa found on the mucous surfaces of man. In this medium all of the amoebae and mucous surface flagellates found in man, with the exception of Giardia lamblia, will survive and multiply and the individual char-

acteristics are better preserved than in any other medium. This medium is prepared from:

(1) Eggs
(2) Sterile Ringer's solution (Autoclaved at 15 lbs. pressure for 20 minutes and then allowed to cool)

Sodium chloride	8.0 grams
Potassium chloride	0.2 "
Calcium chloride	0.2 "
Distilled water	1000 cc.

(3) Sterile horse serum (Must not contain tricresol or other preservatives).

Four whole eggs are emulsified in 50 cc. of the sterile Ringer's solution. Place 4 cc. of this mixture into each test tube and sterilize in an autoclave as follows: Turn the steam into the outer chamber of the autoclave until the jacket is hot, then place the tubes in a slanting position in the sterilizing chamber, close the door and be sure that the vacuum exhaust valve is also closed. Turn the steam into both chambers of the autoclave and open the outside exhaust valve. At the first appearance of steam from this valve close it and allow the pressure to climb to 15 lbs., at which time shut off the steam and (caution) allow the pressure to decline to 0 of its own accord before removing the media. Repeat this procedure on three successive days, storing the media at room temperature between sterilizations. Prior to use, 4 cc. of the liquid portion of the medium, consisting of sterile horse serum one part and Ringer's solution eight parts, are added. The addition of sterile whole wheat powder at the time of inoculation as outlined under St. John's medium will sometimes increase the rate of growth of E. histolytica.

b. St. John's Wheat Broth Medium.

This medium is of differential value in the cultivation of amoebae in that only E. histolytica can maintain growth in it. However, many strains of E. histolytica can not be cultivated in it. The medium is made as follows:

(1) Powdered heart muscle	1 gram
(2) Locke's solution	1000 cc.
Sodium chloride	8.00 grams
Calcium chloride	0.20 "
Potassium chloride	0.20 "
Magnesium chloride	0.10 "
Sodium di-hydrogen phosphate (NaH_2PO_4)	0.10 "
Distilled water	1000 cc.
Sodium bicarbonate	0.40 grams

Mix the above ingredients and boil in a double boiler for one hour, filter through medium filter paper and tube in 10 cc. amounts. Then sterilize in an autoclave at 15 lbs. pressure for 15 minutes.

(3) Whole wheat flour is ground in a mortar until it will pass through a 50 mesh sieve (50 wires to the inch), then tube four gram amounts and sterilize in an autoclave at 15 pounds pressure for 15 minutes, followed by drying in a hot air oven at 58°C. for twenty-four hours.

(4) Just prior to inoculating this medium, take up 0.05 cc. of the sterile whole wheat powder in a clean, sterile, dry, wide bore one cc. pipette and discharge it into the liquid medium by tapping the pipette against the inside wall of the tube.

(5) When amoebae cultured in this medium show signs of degeneration or cyst formation, it is necessary to add 0.5 cc. of sterile horse serum to each culture to revive them.

2. CULTURE TECHNIQUE.

a. Precaution.

Protozoa of this type are quite sensitive to changes in the bacterial flora in vitro; they quickly die in the presence of certain bacteria. Therefore, sterile precautions in sofar as is possible, should always be used in this technique.

b. Routine Cultures.

Using an applicator or wire loop inoculate a portion of the specimen about the size of a pea, consisting of fresh mucus, mucus and blood, or concentrated cysts, into the liquid portion of the medium and thoroughly emulsify it there. Incubate at 37°C. and examine at the end of 24 and 48 hours. Flagellates growing in the medium will be found throughout the liquid portion while amoebae will be found in the very bottom portion of the liquid fraction. The specimen from the culture to be examined for amoebae is obtained by introducing a clean sterile 1 cc. pipette, with the index finger held tightly over the upper end, into the culture so that the tip is at the extreme bottom portion of the liquid medium, then gently release the finger pressure allowing only 1/10 cc. of the material in contact with the bottom to run into the pipette. Resume the finger pressure on the upper end of the pipette and then withdraw it from the culture. The material in the pipette can then be used to transfer the culture, make fresh wet unstained or stained preparations. If it is desired to carry on the positive cultures they should be routinely transferred every 48 hours. However, the survival of these organisms in culture depends a great deal upon the bacterial flora present. One in which E. coli predominates is usually favorable and one in which spore bearers, Pseudomonas seruginosa, or Proteus vulgaris predominate is usually unfavorable. In the latter case transfers should be made at 24 hour periods.

Cultures for amoebae that are not positive at the end of 48 hours should be transferred and further examined as follows: Allow

the culture to remain in the incubator 2 hours, then without unduly disturbing it, remove all but 0.5 cc. of the supernatant fluid by means of a sterile pipette equipped with a rubber nipple. Wash the slant with the remaining fluid and then transfer it to new medium. The resulting culture should then be re-examined at 24 and 48 hour intervals before calling it negative.

c. Method of Ridding Amoebic Cultures of Blastocystis hominis.

(1) Lactic Acid Medium.

Prepare the following modified Locke's solution and sterilize it in an autoclave at 15 pound pressure for 15 minutes.

Sodium chloride	9.0 grams
Calcium chloride	0.24 grams
Potassium chloride	0.42 grams
Sodium bicarbonate	0.20 grams
Lactic acid, 1.0 N.	0.23 cc.
Distilled water q.s.ad.	1000.00 cc.

With sterile precautions, mix 1 part of sterile horse serum with 7 parts of the cool, sterile Locke's solution. Then add 5 cc. of the resulting solution to previously prepared sterile egg slants, i.e., the solid medium prepared for Boeck-Drbohlav medium.

(2) Amoebic cultures containing Blastocystis hominis are treated as follows: Add sufficient sterile 1 to 10,000 neutral acriflavine to Ringer's solution to the supernatant fluid of an amoebic culture on Boeck-Drbohlav medium to make a 1 to 50,000 solution. Incubate at 37°C. for 24 hours, transfer the culture, repeat the process and then transfer the culture to lactic acid medium. Transfers of the lactic acid medium cultures are made at 24 hour intervals for three successive times and then the culture is transferred to straight Boeck-Drbohlav medium. There will still be some Blastocystis hominis present in the culture, but the organisms will usually all be in a degenerated condition and will completely disappear upon successive transfers of the culture. In case they do not, the entire procedure is repeated.

VI. LABORATORY ANIMALS.

Laboratory animals are of no value as a diagnostic procedure is this group However, in the case of E. histolytica, the virulence of this organism can be tested by injecting cultures or freshly isolated trophozoites into the rectum of a young kitten by means of small catheter and syringe. An acute fatal dysentery is usually produced.

SPECIAL METHODS USED IN THE DIAGNOSIS OF THE PROTOZOAN PARASITES AND SAPROPHYTES FOUND ON THE MUCOUS SURFACES OF MAN.

I. THE AMOEBAE.

1. FRESH UNSTAINED WET MOUNT.

The wet fresh unstained mount prepared as outlined under general methods is examined systematically covering the slide without repeating the microscopic fields. Amoebae in the live state have a particular refractive, translucent, granular character which easily differentiates them from other objects in the specimen. This property can be exaggerated and will greatly aid in picking out the amoebae when the slide is being rapidly examined if the high power objective of the microscope is focused slightly above the objects in the preparation. If the observer is well trained, amoebae may be recognized under the low power objective and then a switch made to the high power objective to determine the detailed characteristics. If the outline below is followed in studying amoebae it will be found to be of great aid in species determination.

DIFFERENTIAL POINTS TO OBSERVE IN AMOEBAE IN FRESH SPECIMENS TO DETERMINE THEIR SPECIES.

1. Size and color.
2. Differentiation of ectoplasm and endoplasm.
3. Granularity of endoplasm and presence of cell inclusions.
4. Nucleus - visibility, location when in motion, size.
5. Motility, type (active, sluggish, progressive or non-progressive)
6. Pseudopodia (single or multiple; clear or granular)
7. Flowing of endoplasm into pseudopod (slow or explosive)
8. Presence or absence of (red blood cells in endoplasm; bacteria in endoplasm)
9. Presence of (food vacuoles; contractile vacuoles)
10. Presence and characteristics of chromatoid bodies.

11. Cultural characteristics (growth on R. E. S.
(and St. John's Medium
(on successive transfer

AFTER ADDING LUGOL'S SOLUTION TO MOUNT

1. Nucleus - size, shape, distribution of chromatin, and size and uniformity of granules, location of karyosome.

2. Presence of glycogen bodies.

3. Cysts, if present - character of, size, number of nuclei and type, visibility, glycogen bodies.

2. STAINED SPECIMENS.

Stained specimens should be made of all amoebae where species can not be recognized readily in the fresh unstained state.

3. CULTURES.

Cultures properly made will increase the number of positive findings and allow further study in determining the true species of an amoeba.

4. CONCENTRATION FOR CYSTS.

Concentration methods for cysts should be done routinely on all fecal specimens that are examined.

5. THE COMPLEMENT-FIXATION TEST.

At the present time this test is still in the experimental stage in its development. It probably will become of practical diagnostic significance when a true E. histolytica antigen can be developed. The preparation of such an antigen requires that the organisms be grown in pure culture in the absence of bacteria, and to date this has not been accomplished, or at least has not been reported in the literature.

6. CHARCOT-LEYDEN CRYSTALS.

Charcot-Leyden crystals are indicative of chronic inflammation and are often found in the feces.

7. DIFFERENTIATION OF AMOEBIC AND BACILLARY DYSENTERY.

Differentiation of amoebic and bacillary dysentery can presumptively be done by the general character of the fecal exudate. However, one may complicate the other and amoebic dysentery in the presence of severe bacterial secondary invasion of the ulcers may have many of the characters of a bacillary dysentery. Therefore, every effort should be exerted to demonstrate the causative organism in each case. The main differential points are shown in Table 87.

TABLE 87

DIFFERENTIAL DIAGNOSIS TABLE* ON THE FECAL EXUDATES

IN BACILLARY AND AMOEBIC DYSENTERY

Exudate	Bacillary Dysentery	Amoebic Dysentery
Blood	Varying amounts	Small amounts to actual hemorrhage
Polymorphoneutrophiles	About 90% of exudate. Many show nuclear degeneration (ringing). Cytoplasm frequently contains fat.	Few. Cytoplasm of some of those present shows degenerative changes and in such the nuclei may appear pyknotic.
Endothelial macrophages	Present in varying numbers. Actively phagocytic, frequently contain erythrocytes and leucocytes. Undergo toxic degeneration; "ghost cells".	Not seen except in cases also having bacterial dysentery.
Plasma cells	Present, relatively more abundant early.	Present in small numbers.
Pyknotic bodies	Proportionately insignificant, but are found.	Constitute about 80% of cellular elements.
E. histolytica trophozoites	Absent unless the two diseases are both present.	Present and must be found to make diagnosis.
Amount of exudate, actual hemorrhage excluded	Massive, a large part of the stool.	Small.
Bacterial content	Low	Very high, usually.

* The Cytological Diagnosis of Dysenteric Conditions and its Application in the Military Service. Major George R. Callender, M.O., Military Surgeon, June, 1925.

8. CONFUSING OBJECTS.

a. Animal Tissue Cells.

Animal tissue cells derived from the host or ingested as food may at times appear like amoebae, but carefulexamination of them will easily establish their true nature. Macrophages may be found containing phagocytized red blood cells, but examination reveals their typical metazoan nucleus and as a rule amoeboid movement is never observed. Epithelial cells are pale in color and have metazoan characteristics that easily differentiate them.

b. Vegetable cells.

Vegetable cells such as starch granules, pollen granules, yeast cells or other cells of this type have a certain definiteness of outline and structure which should lead to no confusion. However, yeast cells, such as Blastocystis hominis, may be temporarily confused with cysts of amoebae. The presence of budding forms and the particular structure as illustrated in Figure 59 should cause no difficulty in differentiating them.

II. FLAGELLATES AND BALANTIDIUM COLI.

1. WET FRESH UNSTAINED MOUNT.

The wet fresh unstained mount prepared as outlined under general methods is the usual method of diagnosing these organisms. The movement, shape, size and structure of these organisms usually makes it easy to readily determine their species.

2. STAINED PREPARATIONS.

Preparations stained by Harris' or Heidenhain's method are used for detailed study of the morphological characteristics.

3. CULTURES.

Cultures in R.E.S. medium will sometimes be positive for these organisms when they can not be found in the fresh specimen. Balantidium coli is cultured with difficulty, but these organisms are so large that they can hardly be overlooked; for this reason culture is usually unnecessary.

4. CONFUSING OBJECTS.

Confusing objects are mostly accidental contaminating free-living protozoa which are readily differentiated by the type of nucleus and presence of contractile vacuoles. For details see discussion of these organisms.

REFERENCE BOOKS

1. Blacklock, D. B. and Southwell, T., A Guide to Human Parasitology, Baltimore, the Williams and Wilkins Co., 1932.

2. Boyd, Mark F., An Introduction to Malariology, Cambridge, Massachusetts, Harvard University Press, 1930.

3. Calkins, Gary N., Biology of the Protozoa, Philadelphia, Lea and Febiger Co., 1926.

4. Craig, Charles F., The Malarial Fevers, New York, William Wood and Co., 1909.

5. Craig, Charles F., Laboratory Methods of the United States Army, Third Edition, Philadelphia, Lea and Febiger Co., 1929.

6. Craig, Charles F., Parasitic Protozoa of Man, Philadelphia, J. B. Lippincott Co., 1926.

7. Dobell, Clifford and O'Connor, F. W., The Intestinal Protozoa of Man, New York, William Wood and Co., 1921.

8. Hegner, Robert and Taliaferro, W. H., Human Protozoology, New York, The MacMillan Co., 1924.

9. Manson-Bahr, Philip H., Manson's Tropical Disease, Ninth Edition, New York, William Wood and Co., 1931.

10. Rogers, Sir Leonard and Megaw, John W. D., Tropical Medicine, Philadelphia, P. Blakiston's Son and Co., Inc., 1930.

11. Stitt, E. R., The Diagnostics and Treatment of Tropical Diseases, Fifth Edition, Philadelphia, P. Blakiston's Son and Co., 1929.

12. Thompson, John Gordon and Robertson, Andrew, Protozoology, New York, William Wood and Co., 1929.

13. Wenyon, C. M. Protozoology, New York, William Wood and Co., 1926.

Phylum Platyhelminthes, Claus, 1871.

Class I Cestoda

The Cestodes or tapeworms are endoparasitic and the adult worms are found exclusively in the intestinal tract of the host. The adults of this class are flat ribbon-like, segmented worms with relatively small heads as compared to the size of their mature body segments. The heads bear sucking organs for attachment to the mucosa of the host's intestine. In addition, the head may have a rostellum which in some species is armed with characteristic hooklets. The posterior part of the head of the adult worm is a proliferating area, continuously supplying young segments. A digestive system is entirely absent, nutrition being carried on by direct absorption. All of the worms of this class are hermaphroditic (they contain both male and female generative organs.). The ova usually contain a mass of cells among which are six minute hooks. This indefinitely defined embryo is called an oncosphere or hexacanth embryo. When the mature ova are ingested by a suitable intermediate host, the embryos are liberated in the gastro-intestinal tract and in most species invade the tissue of the host undergoing a period of development and in certain of the species propagation as well. When the tissue of the intermediate host containing the mature embryo is ingested by the definitive host the embryo develops into the adult worm. A single species of animal may be the definitive and intermediate host for some of the worms of this class. Of the tapeworms found in man, D. latus requires three hosts, H. nana requires only one, and all the rest require two. It is of greatest importance to remember that the eggs of H. nana, T. solium, and E. granulosa are infective to man. When he swallows H. nana eggs, both the larva and subsequently the adult worm develop in him; when he swallows T. solium or E. granulosa eggs, only the larval forms, Cysticercus cellulosae or Cysticercus granulosa develops, but not the adult. The larval stages of E. granulosa give rise to hydatid disease while that of T. solium gives rise to Cysticercus cellulosa cysts in the brain, eye, muscle, liver, etc. of man.

Definition of terms.

1. The head of any tapeworm, whether adult or larva, is called the scolex.

2. If present, the terminal disc-like or finger-like anterior extremity of the head is called the rostellum.

3. The entire body of a tapeworm is called a strobila.

4. A proglottid is a segment.

5. Proglottides in which the reproductive organs are not developed or only partly, are said to be immature. Those in which they are fully developed and functioning are called mature, while those with a uterus containing eggs are referred to as gravid. The proglottides nearest to the head are always immature, those midway in the body are usually mature while those making up the posterior extremity are gravid.

6. The genital pore is the external opening of the genital system of a proglottid. It may be single or double and is situated on the lateral sides or center of the ventral surface. If its location is lateral, they may be all on the same side of succeeding proglottides or roughly alternate sides.

7. Oncosphere is the term used in designating the embryo within the egg which has within its cellular mass six miniature hooklets.

8. The ventral surface of a proglottid is that nearest to which the ovary lies. In a majority of the species, this can be determined only by making sections, however, if the genital pore is centrally located, it is on the ventral side.

9. Calcareous corpuscles are highly refractile bodies often spherical, composed of carbonate of lime, and measuring from 5 to 25 u. They are found on or near the surface of the proglottides and scolex.

10. Vitelline glands are glands which supply the nutritive substance or yolk in the formation of an egg.

11. Shell gland. Originally this gland was thought to be the organ supplying the shell for the eggs. It is now known that this is not the function of this gland. Its real function is not known.

12. Sparganum. This is a term applied to all Dibothrio-cephalid larvae (plerocercoids), the adults of which are not known.

Key to Genera of adult cestodes usually found infecting man.

1. Large worms with segments each having one genital pore situated on one of the lateral margins..... Taenia.

2. Large worms with segments each having one genital pore situated centrally on flat surface .....Dibothriocephalus

3. Small worms with segments up to 5 mm. in length and 3 mm. in width, each having two genital pores, one on each lateral margin..... Dipylidium.

4. Small worms, minute, thread-like, segmentation only distinct under low power magnification, segments each having one genital pore situated on one of the lateral margins ..... Hymenolepsis.

Key to species of usual adult cestodes infecting man.

1. Tapeworms belonging to Genus Taenia, uterus with from eight to ten main lateral branches on one side ..... Taenia solium.
Uterus with 14 to 30 main lateral branches on one side ..... Taenia saginata.

2. Tapeworms belonging to Genus Dibothriocephalus. Gravid segments as broad as long, uterus appears as a brownish patch, rosette-like in arrangement in the center of each mature segment.. Dibothriocephalus latus.

3. Tapeworms belonging to Genus Dipylidium ..... Dipylidium caninum.

4. Tapeworms belonging to Genus Hymenopelis - Segments microscopic, one genital pore on each segment and all pores on the same side of the worm, rostellum of scolex armed with single row of hooklets, length of entire worm 25 to 40 mm. ..... Hymenolepsis nana.

5. Tapeworms belonging to Genus Hymenolopesis - rostellum of scolex unarmed, length of entire worm 20 to 60 cm. mature and gravid segments 0.8 to 0.9 mm. wide ..... Hymenolepsis diminuta.

Dibothriocephalus latus (Linnaeus, 1758)

This worm is commonly known as the fish tapeworm due to the fact that infestation is contracted by eating raw, salted, pickled or insufficiently cooked fresh water fish which have been previously infected by the parasite. Man, dog, cat, and fox may act as definite hosts. This parasite is quite prevalent in the area about the Baltic Sea in Europe, also in Russia and Japan. A local focus has been established in the fresh water fishes of the lakes of Minnesota and the adjacent region in Canada by immigrants principally from Finland, Norway, and Sweden. This tapeworm is very long lived (up to 14 years) and sometimes produces a severe anemia not unlike that found in pernicious anemia. The anemia is thought to be produced by hemolysins liberated from the decomposing gravid segments. It not only affects the circulating red blood cells but the blood forming bone marrow as well. There is usually a multiple infection in man. The eggs of this species are operculated and are more commonly found in the feces than those of taenia due to the fact that they are more readily liberated from the uterus.

Morphology.

The worm is usually 2 to 4 meters in length and its maximum width is 1 to 2 cm. The terminal segments are broader than they are long, or are nearly square. There may be as many as 4000 segments in the mature worm. The gravid segments tend to break off, not singly but in short chains. The head or scolex is olive-shaped and has two grooves (bothria) located one on each side. There are no acetabulae or suckers, nor is a rostellum present as is found in the Taenia. The genital pore is located centrally on the flat ventral surface and when viewed microscopically is divided into 3 sections, the anterior opening being that of the vas deferons, the middle one the vagina, and the posterior one the uterus. The testes are very numerous and are scattered throughout the superficial layers of the segment. They cannot be distinguished from the vitalline glands without differential staining. The uterus arises as a straight tube, thin coils to a rosette form, finally opening in the genital pore. In the gravid segments it appears as a brown, centrally located mass. The eggs are operculated, yellow to golden brown in color, and measure about 70 to 45 M. They can thus be differentiated from the operculated fluke eggs of Fasciolopsis buski and Fasciola hepatica which are about 150 M. in greatest diameter. The eggs, when passed in the feces, contain an unsegmented ovum surrounded by yolk. When the feces containing the eggs is diluted with water a hexacanth embryo develops in from 3 to 5

weeks depending on the temperature. Under these conditions, cilia appear and entirely cover the embryo or embryophore as it is designated.

The operculum opens and the ciliated embryophore enclosing the oncosphere, escapes and is then called a coracidium. The coracidium swims about freely by means of its cilia and unless it is ingested by its first host, a crustacean Cyclops streniun or Dioptomus gracilis, it dies. Within the crustacean host, the cilia are lost and the larvae migrate into the coelomic cavity where it undergoes further development and then is called a procercoid. After a period of 9 to 10 weeks, it is 600 to 700 M. in length and is now infective for certain species of fresh water fish (pike, perch, grayling, etc.) if they should ingest the infected crustacean. The procercoid larva is liberated from the crustacean by the digestive juices of the fish and then migrates through the intestinal wall of the fish, coming to rest in the fat of the mesentery or in the muscle tissue. It is now called a plerocercoid, and after a period of development it reaches a size of 1.5 cm. in length and 2 mm. in width. It is grayish white in color and ribbon-like in shape. It is not destroyed by ordinary salting, pickling, or smoking.

It is infective for man, dog, or wolf, and if poorly smoked, raw or insufficiently cooked fish are eaten, the plerocercoid larva is liberated in man's gastro-intestinal tract and attaches itself to the mucosa and is now a true scolex. It gives rise to a chain of segments forming the mature worm. During the early developmental stages it sometimes increases in length by as many as 30 segments daily. Within a period of 3 t 8 weeks from the time of ingestion, mature segments and ova will be passed in the feces.

Laboratory diagnosis -- finding of characteristic operculated ova or proglottids in the feces. There is usually a relative eosinophilia (8 to 15%). Anemia with an increased color index may or may not be found.

Sparganum proliferum (Ijima, 1905), Stile, 1908

This larval parasite has been found in certain districts of Japan and in Florida. The adult form is unknown, but the plerocercoid larval forms occur in large number in cysts in the subcutaneous tissues of man. These cysts measure about 3 to 12 mm. in length by 300 M. in width. The larva are narrower anteriorly but do not contain grooves as they are not fully developed. These plerocercoid larvae proliferate by budding so that in the older cysts there may be as many as seven larva present. The cysts feel like rice grains or shot in the subcutaneous tissue and give rise to a severe acne-like condition, with oedema of the surrounding tissue and regional lymph gland enlargement. Occasionally deeper structures are involved by the cysts.

Laboratory Diagnosis. Biopsy with isolation of the plerocercoid larva from the cysts which, if observed, are usually motile.

Taenia saginata (Goeze, 1758.)

This is the beef tapeworm and is the most common large tapeworm of man. It is world wide in its distribution and is contracted by th ingestion of insufficiently cooked beef which contains the live

Cysticercus bovis (larva). Any person harboring this parasite in his intestinal tract is soon aware of its presence due to the fact that the gravid proglottids become detached and are motile. They sometimes migrate out of the rectum singly or rarely in short chains and are then discovered in the under clothing. If passed in the feces they usually show motility and will be seen making undulating, progressive serpentine movements about the fecal mass. They apparently are seeking to leave the fecal mass as far as possible before disintegrating and liberating the mature ova. This characteristic would enhance the possibilities of their being ingested by cattle due to their distribution over a wider area. The motile gravid segments are sometimes mistaken for flukes but careful examination readily establishes their true identity.

Morphology.

The worm usually contains more than 1000 segments and measures 3 to 4 meters in length. The strobilla tends to be pleomorphic. There is considerable variation in size and shape of comparable proglottids and at times of the scolices between different individual parasites of this species. The scolex is about 1.5 to 2 mm. in diameter and does not contain a rostellum or hooklets. Four round to oval acetabulae or suckers are symmetrically located about the head and serve as the sole organs of attachment. The neck is about one half the width of the head and twice as long. The gravid proglottids are about 5 to 7 mm. in breadth by 20 mm. in length. The genital pores are lateral in position and roughly alternate sides. The ovary has only two lobes and the uterus in the gravid proglottids is an elongated central longitudinal tube with 14 to 30 compound dendritic lateral branches on each side. In such segments the other reproductive organs have atrophied and the remaining structure is nothing more than an egg sack. The eggs are ordinarily indistinguishable from those of T. solium but if the embryonic membrane is still present it has two delicate polar processes which are not found on similar ova of T. solium. The eggs are 30 to 38 M. in diameter and are usually pale buff in color. The outer membrane or shell is a thick walled structure perforated with minute pores which on examination under the microscope give the impression of radial striae. Within the shell is a fully developed embryo (onchosphere) with three pair of clearly distinguishable hooklets.

Life history.

Man is thought to be the only definitive host of this parasite. When the eggs or gravid proglottids are passed in the feces of man and subsequently contaminate forage, water or pasture land, the ova may be ingested by cattle or in rare instances by closely related herbiverous animals, (buffalo, giraffe, and llama). Within the gastro-intestinal tract of its intermediate host the larva emerges from the shell of the egg and penetrates the intestinal wall, gains entrance to the lymphatics or blood stream and then finds its way to the connective tissue areas in striated muscle. The most common side of localization is the pterygoid, tenderloin, tongue and heart muscles. When localized in the connective tissue of muscle the larva develops into a cysticercus (bovis) which is commonly called a bladder worm. The larva is an egg shaped milky white object, frequently translucent and measures 7 to 10 mm. in breadth by 4 to 8 mm. in length. Within the bladder is an invaginated miniature

scolex characteristic of this species. In infected beef the Cysticercus bovis larva can easily be seen; between the fibers of the muscle proper, near the tendinous insertion or near the fascial planes. Cattle that become infected with this tapeworm larva, if not reinfected, will destroy the larva within a period of one year. The only observable remnant of the larvae after that time is a calcified or caseous mass.

If the live Cysticercus bovis larvae are ingested by man, (this is frequently the case when rare beef that has not been carefully inspected is eaten),* the scolex evaginates itself when the cystecercal wall is digested away and attaches itself to the gut wall where it begins to grow. Eight to 12 weeks from the time of attachment gravid proglottids and ova are being passed in the feces.

*U. S. inspected beef is a safe product to use in that the inspections are carefully made and in addition storage under refrigerated conditions with the temperature low enough and the time long enough to kill any of the larvae that may have been missed by the inspection. Storage, fresh or pickled, under refrigeration, 36° F., for 3 weeks, or 15° F. or lower for 6 days.

Laboratory Diagnosis. The findings of large gravid proglottids with a single lateral genital pore and with 14 to 30 lateral branches to the uterus. To visualize the uterine branches fix the proglottid in hot (70° C.) 3% formalin for 5 minutes and then place it between two slides. If the mounted specimen is held up to the light, the uterine branches will stand out as white lines extending out from the central stem. There is usually no differential increase in eosinophiles.

Taenia solium (Linnaeus, 1758).

This is the pork tapeworm, and is sometimes designated as the armed tapeworm due to the fact that the rostellum is armed with a double row of hooklets. It is world wide in its distribution and may be found wherever raw or insufficiently cooked pork is consumed. It is most prevalent in Europe and certain sections of the tropics but is a relatively rare infection in the United States. This infestation iscontracted by ingestion of live cysticercal larvae in pork that has been insufficiently cooked. These larvae are not killed by cold storage and are resistant to even freezing temperatures. Smoking or pickling does not kill them but they are readily killed by ordinary cooking temperatures. The eggs of this parasite are also infective to man, and if ingested, the larva upon being freed from the egg shell invade man's tissue and form cysts (Cysticercus cellulosa) in the muscles and organs of the body.

Morphology

The adult worm is usually made up of from 600 to 800 segments and measures from two to three meters in length. It is usually smaller than T. saginata. Malformed segments or scolices are not uncommonly found in specimens of T. solium. The scolex is about 1 mm. in diameter and is roughly quadrate in shape. It may rarely show some pigmentation. A

rostellum is present and is armed with a double row of alternating large and small hooklets numbering as a rule from 22 to 32. Four protuberant cup-shaped acetabulae or suckers are symmetrically arranged in the same plane about the head. They, with the rostellum, serve as the organs of attachment. The neck is about one-half the diameter of the head. The immature and mature segments are usually longer than broad and are strikingly like those of T. saginata, differing only in minute detail. The ovary which lies in the posterior portion of each mature segment has 3 lobes, a symmetrical pair and an accessary lobe on the side containing the genital pore. (T. saginata has only a two-lobed ovary). The uterus in the mature segments is a central longitudinal tube with 8 to 10 lateral dendritic branches on each side. T. saginata has 14 to 30 branches to a side.

The eggs are ordinarily indistinguishable from those of T. saginata unless the embryonic membrane is still present. If present it does not have delicate polar processes as does T. saginata.

Life history. The adult stage of this parasite is found only in man and there is usually only a single parasite present. Several gravid segments are usually passed in the feces together as a short chain. They usually show sluggish motility but as a rule do not have enough power to migrate from the rectum of their own accord. When the eggs of gravid proglottids are passed in the feces of man they remain in the area of the fecal deposit and if ingested by other animals (principally pigs -- rarely man, sheep or dog), the larval stage is liberated from its shell by the digestive juices and then penetrates through the mucosa and invades the blood or lymph stream where it ultimately lodges in muscle, or some organ of the body, and develops into a cysticercus. This cysticercus larva (called Cysticercus cellulosa) develops until it is oval in shape and about 5 x 10 mm. in size. It has an opalescent, grey, milky, translucent color. It is readily visible to the naked eye and when observed in muscle, imports an appearance known as measly (pork).

Cysticercus cellulosa infections in man are ordinarily the result of the ingestion of eggs of T. solium through either contaminated food or water. It is possible for a person infected with the adult T. solium to ingest the eggs through contamination of his hands and mouth parts from his own fecal excrement. It is the opinion of some helminthologists that auto-infection sometimes takes place due to gastro-intestinal upsets with reverse peristalsis. Eggs are carried from the intestines up into the stomach where action of the digestive juices on the egg shells cause the larvae to be liberated, whereupon they invade the tissue and eventually lodge in muscle or organs of the body, giving rise to Cysticercus cellulosa disease.

When insufficiently cooked pork contaminated with live Cysticercus cellulosa larvae is ingested by man, the cyst walls are digested away and the miniature armed scolices evaginates and then attaches itself to the intestinal wall. Development of the adult worm then begins and within a period of from 5 to 12 weeks adult segments and eggs are being passed in the feces. The adult worms according to Faust may live as long as 25 years in the intestinal tract of man. Any person infected with T. solium is of great danger to any other person in contact with him or his surroundings due to the danger of contracting Cysticercus cellulosa

infections. As soon as diagnosed he should be isolated until rid of the parasite and his fecal excrement should be disposed of by burning or thoroughly mixing with a 10% cresol solution.

Laboratory Diagnosis.

1. The finding of large tapeworm proglottids with a single lateral genital pore and 7 to 10 lateral branches of the uterus in the feces of man.

2. If the proglottids are stained and cleared, a three-lobed ovary can be demonstrated.

3. Following treatment the recovery of a large tapeworm with an armed scolex.

Echinococcus granulosa. (Taenia echinococcus) (Batsch-1786, Rudolphi,1805)

This is the tapeworm, the larval stage of which causes hydatid disease of man. Hydatid disease is world wide in its distribution, principally among people closely associated with livestock and dogs. Dog, wolf, jackal, and cat are the definitive hosts of this parasite, while sheep, cattle, horses, goats, pigs, and rabbits are the intermediate or larval hosts. This parasite is quite common in Australia, Russia, Iceland and temperate South America but is rare in the United States. McGath of the Mayo Clinic, in reviewing all of the reported cases in the United States (482) doubts if more than 5% of the cases of human infection with the larvae of this parasite were contracted in the United States. All other cases were either immigrants or people who had spent some time in an endemic area elsewhere.

Morphology.

Echinococcus granulosa is a minute tapeworm and is usually not over one (1) centimeter in length. Unlike other tapeworms the strobila consists of a head and only three to four proglottids of which one is immature, one or two are mature and only one (the terminal segment) is gravid. The head is piriform in shape and is 300 M. in diameter. A rostellum is present and is armed with a double row of 28 to 50 hooklets. Four symmetrically placed oral acetabulae or suckers are present. The neck tapers backward from the head and is narrowest just anterior to the first segment. The second segment is mature and shows fully developed sexual organs. All segments are broader than long and the terminal gravid segment contains a uterus which because of lateral evaginations gives the appearance of a loosely twisted coil. When the uterus is filled with eggs it bursts open and the ova are liberated. This may occur before or after the gravid segment is detached from the remainder of the worm. The eggs when liberated are surrounded by an embryonic membrane which soon disintegrates. The eggs as found in the feces of dog are indistinguishable from that of T. solium or T. saginata.

Life History.

Dogs infected by this parasite usually harbor a great number of the mature worms in their intestinal tract. When passed in the feces, the

eggs are scattered with the fecal excrement and contaminate pasture land, forage, or water. There they are usually ingested by their most favorable intermediate host (sheep). (Man or any of the herbiverous animals may act as intermediate hosts). When the eggs reach the intestinal tract they break from their shell and penetrate the mucosa and gain entrance into the blood stream where they are carried to various parts of the body. The greatest number land in the liver. A lesser number in the lungs and scattered individuals in the remainder of the organs and tissue. By the end of three weeks a larval cyst 250 M. in diameter is formed by each parasite surviving, and the body of the host is beginning to form a defensive wall of scar tissue about the parasite. By the end of 5 months the cysts are 1 centimeter in diameter and now show differentiation into an outer cuticular elastic fibrious layer and an inner germinal layer. Cysts like evaginations of the germinal layer are now protruding inward into the fluid contents of the cyst. These soon show invaginations with the formation of scolices. These daughter cysts at times become detached and eventually may give rise to granddaughter cysts. Thus a great number of scolices are produced from one original larva. These granular scolices are sometimes designated as hydatid sand. They usually are all alive and infective. If the cysts are ruptured, the scolices will scatter through the tissue and give rise to new cysts. In addition, the fluid contents of the cysts are quite toxic and may cause shock if taken up by the circulation. The primary cyst formed by the larvae may develop into a simple unilocular cyst or at times into a multilocular cyst. The multilocular cysts are thought by some helminthologists to be due to a separate and distinct species of E. granulosa.

If raw meat containing hydatid cysts is ingested by dogs, the scolices are liberated by the digestive juices and develop into mature worms within a period of three weeks.

Laboratory Diagnosis. - (Of hydatid disease)

1. Intradermal test. 0.2 cc. of a sterile hydatid fluid is injected intracutaneously, a wheal with an out erythematous zone appears within 15 minutes and both gradually fade, followed by a delayed secondary rise several hours later.

2. Precipitin test.

3. Complement fixation test.

4. X-ray.

5. Surgical removal of hydatid cyst followed by histological examination.

Hymenolepis nana. (v. Seebold, 1852 - Blanchard, 1891).

This is the dwarf tapeworm and is the most frequent of all tapeworms encountered in man. It is world wide in its distribution and is frequently found in the United States and its possessions. It is closely related or identical with the dwarf tapeworm of rat and the mouse. (Hymenolepis nana vir. fraterna).

Morphology.

The strobila has a length of only 25 to 40 mm. and its maximum diameter does not exceed 1 mm. The head is rhomboid in shape with a maximum diameter of .32 mm. There are four acetabulae or suckers present and a rostellum armed with a single row of hooklets numbering 20 to 30. The neck is long and slender and the proximal segments are very narrow and short. The mature and gravid segments are three to four times as wide as long. The strobila usually contain about 200 segments and the largest gravid ones are about 0.03 mm. in length by 0.9 mm. in width. The gravid segments drop off from the worm one by one and then rapidly disintegrate, liberating the ova. Gravid segments are seldom found in feces. The eggs are 30 to 45 M. in diameter and are spherical or slightly oval in shape. The eggs as seen under the microscope show an outer shell and two inner membranes surrounding the onchosphere or embryo. The outer membrane has two diametrically opposite polar thickenings, from each of which arise 4 to 8 thread-like filaments. (These polar thickenings and filaments are not present on the only other helminth egg (Hymenolepis diminuta), that resembles that of H. nana.) The onchosphere contains three pairs of lancet shaped hooklets.

Life cycle.

The gravid proglottids disintegrate in the fecal contents of the host's gut and liberate the eggs which, when passed in the feces, may eventually contaminate food or water. Sometimes through carelessness on the part of the individual infected, the hands may become contaminated with the eggs and by this means carried directly to the mouth. All eggs as passed in the feces are infective for man, and when ingested and subjected to the action of the digestive juices the larvae emerge from the shell and penetrate the mucosa of the terminal portion of the ileum where they round up and form cysts within the villi. The cysticercus thus formed is called a cercocyst. After a period of development the cyst ruptures into the gut contents liberating the new formed scolex which attaches itself to the mucosa. Within the period of two or three weeks the worm has reached maturity and is liberating eggs in the feces.

Laboratory diagnosis.

The finding of typical hexacanth eggs in the feces, with outer shell and two inner membranes, the outer membrane having polar thickenings and filaments, in the feces.

Rare species of Cestodes found infecting Man.

The adults of Dypilidium caninum, (common dog tapeworm), Hymenolepis diminuta (the common tapeworm of the rat and mouse), Taenia confusa (life history unknown) and various species of Davainea (the common tapeworms of birds and fowls), are occasionally found infecting man. The larval forms of Multiceps multiceps (European dog tapeworm) and Diphylobothrium mansoni (Sino Japanese dog tapeworm) rarely infect man. The incidence of these infestations is so small in the United States that for practical purposes they need not be extensively studied. They will be briefly

discussed or demonstrated in class if further information is desired, text books such as Faust's "Human Helminthology" or Baylis "Manual of Helminthology" should be consulted.

Phylum Nemathelminthes Vogt (fide Carus, 1863),

The round worm parasites of man are found under the Class Nematoda Rudolphi, 1808. They are in general unsegmented worms bilaterally symmetrical, with three body layers and elongated rounded or filiform bodies. An alimentary tract is present with a mouth and usually an anal opening. The coelomic cavity is large and no cilia are present during any stage of the life cycle. The sexes are usually separate.

Definitions.

Buccal vestibule - The cavity of the mouth.

Cervical and caudal alae - Ridge or wing-like expansions of the cuticle.
Cervical papillae - Minute fingerlike protuberances of the cuticle in the vicinity of the esophagus.
Chitin - A hard transparent substance forming teeth or cutting plates.

Cuticle - The outer dense translucent covering of the worm; corresponds to epidermis in mammals.

Cloaca - The cavity in male nematodes into which the intestines and vas deferens discharge. It is absent in females.

Copulatory bursa - A strong wing-like clasping organ used in copulation.

Copulatory spicule - The copulatory organ.

Dentiguous ridges - Chitinous prominences with serrated or toothed margins.

Gubernaculum - A chitinous elevation in the dorsal wall of the cloaca which acts as a guide for the copulatory spicule during copulation.

Filariform - Applied to the esophagus of a larval nematode when it is long compared with the length of the larvae and does not have a posterior dilated bulb. Larvae having such an esophagus are designated as filariform.

Rhabditiform - a name applied to the esophagus of a nematode larvae when the posterior end is dilated into a bulb. Larvae having such an esophagus are designated as rhabditiform.

Oviparous - A female that lays eggs.

Parthenogenic - A female that produces young without fertilization by the male.

Viviparous - A female that gives birth to living larvae. (Hatching of the eggs having taken place in the uterus.)

Ascaris Lumbricoides Linnaeus, 1758.

This is one of the most common helminths infesting man. It is world wide in its distribution and is particularly prevalent in certain sections of the tropics. It is a very common human infestation in the Philippines, Porto Rico, Panama and to a lesser degree the southern United States. In general it is prevalent in any country within the 60 degrees Fahrenheit isotherm where there is adequate moisture and improper disposal of human excrement. It is thought by many to be identical with ascaris found in hogs, however, human experiments conducted by Japanese investigators (Koino 1922) seem to disprove this assumption. The sexes are separate and the females are about twice as long and one and a half times as great in diameter as the males. The eggs of this parasite are extremely resistant to chemicals and to a lesser extent to desiccation. They are readily destroyed by ordinary cooking temperatures, and are inhibited in their development by temperatures below 40 degrees F. Freezing, however, does not destroy them, they merely remain dormant until favorable temperatures are encountered. Eosinophilia, urticaria, intestinal discomfort and obstruction in some form are quite commonly encountered in individuals infested with this parasite. Infestations are usually multiple although occasionally only a single parasite may be present.

Morphology.

The females of this species are usually 20 to 45 cm. in length and about 3 to 6 mm. in transverse diameter while the males measure from 15 to 25 cm. in length and 2 to 4 mm. in transverse diameter. They are pinkish to yellow grey in color and give the impression of trans lucent outer wall with an inner cavity filled with thread-like organs. On each side a greyish white cuticular line extends the whole length of the worm. The heads of the male and female are identical and are made up of a mediam dorsal broad elliptical lip and a symmetrical pair of submedian ventral lips all of which show fine teeth upon their exposed surfaces. Each lip has on its lateral margins a pair of minute papillae. There is a small buccal vestibule and a cylindrical muscular esophagus 10 to 15 mm. long leading directly to the mid intestine. The intestines terminate in a rectum which opens directly into the anal pore of the female and cloaca of the male. The posterior extremity of the female is straight and tapers to a point while that of the male is curved ventrad. Two retractile club shaped copulatory spicules are usually visible in the curved portion of the posterior extremity of the male and there are several preanal and postanal papillae. Further details of the anatomical structures of these worms will not be discussed because they are of no practical importance in routine identification.

Life History.

The mature female ascaris discharge their fertile eggs into the feces. If males are also present in the gut the female will have been fertilized prior to liberation of the eggs. However, in light infestations with this parasite, males or females alone may be the only worms present. In such an event the eggs, if present, will be unfertile. It is estimated that the genital tubules of a mature female ascaris have a

capacity of 27 million eggs. The average daily output of eggs from each female is about 200,000 (Brom and Cort, 1927).

The fertile eggs as passed in the feces are yellowish brown in color, oval in shape, with a thick transparent shell and an outer coarsely mammillated albuminous covering which is sometimes absent. They measure 45 to 75 M. in length by 35 to 50 M. in their lesser diameter. Unfertilized eggs are usually much longer and normally have a thin irregular mammilated outer shell, filled with an unorganized mass of highly refractive granules.

When the eggs are passed in the feces the embryos are undeveloped but if exposed to a temperature of 91 degrees F. under humid conditions they will develop into rhabditiform sheathed larvae within a period of 9 to 13 days. After this stage of development has been reached they are infective if ingested by man and will readily hatch after passing into the intestinal tract. Under conditions that exist in tropical and subtropical countries the period of development into rhabditiform larvae is usually about one month. After the rhabditiform larvae have once developed in the eggs they are extremely long-lived and may be still alive and infective if kept in moist soil for a period of 5 to 6 years. They will still be alive and motile within the egg after a period of two years in 2% formalin.

Eggs containing live rhabditiform larvae, if ingested by man, pass into the intestinal tract where they hatch and shed their sheath. The rhabditiform larvae are thread-like in shape, tapering at both ends and measure 0.2 to 0.3 mm. in length by 13 to 15 M. in transverse diameter. The larvae penetrate the mucosa and gain entrance into the blood or lymph streams. They are carried by the circulation to the lungs where the majority of them lodge and undergo a further period of development until they are 16 to 21 mm. in length. Their stay in the lung is marked by a pneumonic reaction on the part of the host and in heavy infections the lungs may be almost completely consolidated as in lobar pneumonia. There is usually a marked (12 to 30%) eosinophilia at this stage. After this period of development the larvae migrate via the bronchi, trachea, epiglottis, esophagus and stomach to the intestines where after 6 to 8 weeks they develop into mature worms and the females begin discharging eggs in the feces. The period of exposure to development of mature worms with liberation of eggs in the feces is 2 to 2½ months.

Laboratory diagnosis.

1. Finding of typical mammilated eggs in the feces.

2. Recovery of the large adult round worms following treatment.

Enterobius (Oxyuris) vermicularis (Linnaeus, 1758) Leach, 1853.

This is the pinworm or seat worm of man and is world wide in its distribution. It is encountered particularly in children and individuals with careless uncleanly habits. Due to the fact that the eggs are infective when liberated by the female pinworm and do not require an

extrinsic incubation period, occasional infestations are found in families of the upper social strata. Because of the infectiousness of these eggs and the fact that they will live for about 10 days under conditions of desiccation that exist in the average American home, an infestation once contracted is very difficult to get rid of. If one member of a family is infested all including other intimate contacts will be. Contaminated bath tubs, wash basins, towels, wash cloths and food or dishes are the most frequent sources of transmission from one individual to another. Examination of vacuum cleaner sweepings from a house in which the individuals are infested with E. vermicularis will reveal, as a rule, many viable eggs of this parasite. Pinworm infestations are quite common among married enlisted men and their families but are relatively uncommon among soldiers, with 1 year of service or over, living in barracks. This is due to the fact that married soldiers usually live at home where there is no supervision of sanitation while soldiers in barracks are under strict sanitary control.

Surveys of Washington orphanages, schools and lower working class families by Hall and his associates (1936) have shown that from 12 to 16% are infested with pinworm. Clinical symptoms frequently observed are vaginitis, rectal pain, gastro-intestinal upsets, depraved appetities, constipation, insomnia, nervous irritabilities and loss of weight. Heavy infestation with pinworm can be as damaging to physical and mental development of children as light hookworm infestations.

Morphology.

Males and females of this species have the same type of head. It is slightly bulbous in shape and is made up of three labis which serve as the organs of attachment to the mucosa. The labis are capable of being retracted into the body. The posterior portion of the esophagus is dilated into a bulb and the digestive tract is a straight tube. The females are fusiform in shape and about 8 to 13 mm. long by 0.3 to 0.5 mm. in transverse diameter. The caudal extremity of the female tapers to a fine point and makes up about 1/3 the total length. That of the male is sharply curved ventrad, is somewhat bulbous in shape, and near its terminus is located a retractile, conspicuous copulatory spicule 70 M. in length with an acutely curved tip. The anus of the female opens at the junction of the middle and posterior thirds of the body, while the vagina opens at the juncture of the anterior and middle thirds.

Life cycle.

The adult worms live in the coecum, appendix and adjacent parts of the colon and ileum. After fertilizing the female, the male dies and is passed in the feces. The female in a short time becomes distended with ova, releases her hold on the mucosa and passes with the fecal currents to the rectum. She does not liberate her eggs into the feces but migrates by active crawling through the rectum onto the perianal region or even into the vagina if the host is a female. This migration usually takes place at night but may occur at any time. It is usually accompanied by an intense itching that gives rise to scratching. When the females are upon the perineum they rapidly become desiccated and are either mechanically crushed by the hosts scratching or literally explode due to drying, thus liberating the embryonated eggs, all of which are mature and

infective. The eggs usually are picked up by the fingers or fingernails of the host while scratching the perineum and are carried eventually to the mouth where they are ingested. Eggs not picked up by the fingers of the host continually drop off into the clothing contaminating the area frequented by the individual. This especially is true in bathrooms, bedrooms and bed clothing. These eggs will remain alive and infective for at least 7 days but are usually not viable after 10 days.

The eggs as freed by the female pinworm are oval in shape with one side slightly flattened. They are 25 by M. in size and made up of a transparent outer albuminous shell and an inner embryonic membrane surrounding a well developed embryo.

When the egg is ingested it passes to the small intestine and hatches into an embryo 140 to 150 M. in length. After a short period of development it passes to the coecum where it attaches itself to the mucosa and, within a period of three weeks from the time of ingestion, is mature and ready for egress from the bowel. Reproduction does not take place within the human host and if reinfection could be stopped a person would be free from the parasite after the one generation (3 weeks).

Laboratory Diagnosis.

1. Demonstration of typical eggs from perianal swabbings.

2. Finding of adult pinworms on the perineum or in the feces.

Trichinella spiralis (Owen, 1835) Railleit, 1895.

This is the worm, the larval stage of which gives rise to the disease known as trichinosis in man, hog, rodents, and carnivorous animals. Infection with the larvae of this parasite is cosmopolitan in its extent both for man and animals. In most instances man contracts this infection by the ingestion of insufficiently cooked pork containing the live larvae of T. spiralis. Autopsy study of human cadavers in Boston, Buffalo, Rochester, Minnesota, Washington, D. C., New Orleans, La., and San Francisco show an incidence of infection varying from 3 to 25%. As embryos and not free eggs are liberated by this parasite, it is impossible for man or animals to contract this infection without eating meat containing the live larvae. The majority of hogs probably contract the infection by the ingestion of uncooked pork scraps in garbage. Rarely the ingestion of infested rats may be the source. It has been estimated that from one-half to 15% of hogs are infected with the larvae of T. spiralis, the greater percentage being among hogs fed on garbage from large cities. Garbage fed hogs are indiscriminately slaughtered with grain fed hogs and as their meat is inferior to grain fed hogs they are more often used for sausage making. If pork is cheaper than beef it is often mixed with beef in preparing hamburger meat for use at roadside cafes, restaurants and quick lunch stands. A rare hamburger in such a place stands a good chance of containing live trichina larva. Meat inspection cannot eliminate trichina infested pork from being sold. However, if no raw pork is consumed and if a cooking temperature of at least 137° is reached in all parts of the meat being cooked all the larvae will be killed. Pork used in the preparation of sausages which are meant to be consumed without

further cooking is considered safe if it has been stored at a temperature not exceeding 5° F. for 20 days.

Morphology.

The female worm measures 3 to 4 mm. in length and about 60 to 75 M. in its greatest transverse diameter. It is an elongated round tapering worm having its smallest diameter at the oral end and greatest diameter at the caudal extremeity. The anus opens at the posterior tip and the vulva near the anterior 1/5 of the body. The ova are formed in the ovary near the caudal extremity and by the time they have reached the vulva have become embryonated and hatched. The larvae as liberated from the vulva are 90 to 100 M. in length and 6 M. in diameter and are capable of passing through the capillary beds of the liver and lungs once they gain entrance to the circulation of their host.

The males measure from 1.4 to 1.6 mm. in length by 40 to 50 M. in diameter. They are attenuated anteriorly and slightly bulbous posteriorly. The cloaca opens at the posterior extremity and is guarded by two conical papillae. It is evertible during coitus.

The encysted larvae are found tightly coiled in fibrous or calcified cysts measuring 0.8 to 1 mm. in diameter in or near the tendinous insertion of striated muscle.

Life Cycle.

When live encysted trichina larva are ingested by a suitable host the digestive juices dissolve the cyst capsule and liberate the larvae. The larvae then pass into the small intestines and attach themselves to the mucosa where further growth takes place. By the end of the 3 to 5 days development is nearly complete. The males fertilize the females and then die. The viviparous females then migrate further into the mucosa and eventually come to lie in one of the lymph channels or partially in a small venule. By the end of seven to twenty days embryos are being discharged into the blood stream, from 1000 to 1500 embryos are liberated by each female.

The small motile larvae pass readily through the liver and lungs to the arterial circulation where they are carried to the capiallary beds of the muscles. These larvae triple their size and then encyst near the tendinous insertion of the muscle fibers. They are present in greatest numbers in the muscles of mastication, respiration and phonation, although they may be found in any striated muscle. They may be found in this position as early as 9 days following the initial infection and will continue to increase in numbers present up to the 40th day. During the period of invasion, fever, leucocytosis, eosinophilia (40 to 60 present) muscle pain, nervous irritability and sometimes meningitis symptoms may be present.

Laboratory Diagnosis.

1. Demonstration of encysted trichina larvae in:
   (a) Biopsied muscle or muscle from cadavers.
   (b) X-ray demonstration of encysted calcified larvae.

Trichocephalus trichiurus (Linnaeus, 1771) Blanchard, 1895

This is the humanwhip or thread worm and is quite common in warm moist climates although it may be found elsewhere. It is quite common in the United States and possesseions. This worm although very persistent when once established in the large gut of man does not seem to give rise to nearly so much damage as the other helminths. The whip worms of animals (dog, sheep, etc.) are not known to be infective for man.

Morphology

In shape and general appearance this worm resembles a whip. Its anterior 3/5 is hair-like and corresponds to the lash while the posterior 2/5 is thick and fleshy like the stock of a whip. The males and females are nearly the same size, 130 to 150 mm. in length, but sometimes the males are slightly smaller. The anterior portion of the worms is nothing but a capillary tube which it buries beneath the mucosa parallel to the long axis of the host's gut. The posterior free part of the worm contains the digestive and generative organs, the anus opens at the caudal extremity while the vulva opens at the junction of the bulbous and thread-like portion of the body. The caudal tip of the male is curved ventrad and ends in a copulatory spicule with a retractile thickly spined sheath. The eggs are brown in color, barrel or lemon-shaped with mucous plugs in either end. They measure 50 x 25 M. and contain an unsegmented ovum when passed in the feces.

Life Cycle.

The fertile unsegmented eggs are passed in the feces and are not infective until the embryo has developed. A period of incubation varying from 12 to 60 days depending upon the temperature, oxygen supply and humidity is usually required. The eggs are extremely resistant to drying and low temperature changes. They are said to retain their vitality for 5 years in contaminated soil.

Man is infected by ingesting food or water contaminated with the eggs containing mature developed larvae. The larvae are liberated from the shells by action of the digestive juices and after a short period of development in the small intestine migrate to the caecum and attach themselves to the mucosa, developing into adults within 6 to 8 weeks.

Laboratory Diagnosis.

The finding of characteristic eggs in the feces.

Strongyloides stercoralis (Bavay, 1876) Stiles and Hassall, 1902.

The geographical distribution of this worm is cosmopolitan, being most commonly found infesting man in moist warm climates. Strongyloides infestations of animals are not identical in specie with that of man. Dogs may be experimentally infested with man's parasite but the infestation does not last. Man is probably the only host reservoir for

S. Stercoralis. It is not an uncommon infestation in the United States and its possessions. It is unusual in that a free living generation may form part of the life cycle and that only parthenogenetic females and rhabditiform larvae are found in the small intestines of man or dog. If the infestation is heavy and frequent reinfestation taking place, a persistent bloody diarrhea with urticarial skin reactions will frequently be present, but in light infestations vague intestinal discomfiture is the only thing complained about.

Morphology.

The parthenogenic females measure about 2.2 mm. in length and about 50 M. in transverse diameter. They are so small that they can only be discerned by the use of low power magnification. They live attached to or in the mucosa of the jejunum and upper part of the ileum and can only be demonstrated by the post-mortem scraping of the mucosa using a dissecting scope to separate them from the tissue. They have a cylindrical esophagus which extends one-third of the body length and the vulva opens at the junction of the middle and anterior thirds of the body. In gravid worms the eggs, about 20 to 30 in number and resembling those of hookworm, can be seen lined up in a single row in the uterus extending from near the anus to the vulva. When the eggs are liberated from the vulva they contain a rhabditiform larvae which almost immediately hatches and passes into the feces.

The rhabditiform larvae as found in freshly passed feces are actively motile and about 225 M. in length by 16. M. in breadth. They have a shallow buccal vestibule as compared with hookworm larvae and are less attenuated posteriorly. The esophagus is typically rhabditiform in type with a short club-shaped anterior portion and a globular bulbous dilitation posteriorly. The esophageal bulb is larger than that found in the rhabditiform larva of hookworm. The free living adult resemble the rhabditiform larva in type, the male measuring 0.7 mm. in length by 45 M. in width. The female 1 mm. by 50 M. The caudal extremity of the male is curved ventrally and bears two minute brown curved copulatory spicules about 40 M. in length. The position of the uterus, vulva and eggs in the free living female is similar to that of the parthenogenic female. The filariform larvae are about 500 M. in length and differ from the filariform larvae of hookworm in that the esophagus extends to about one-half the body length and the tip of the tail is slightly notched.

Life Cycle.

The parthnogenic female liberates her embryonated eggs on the mucosa of the small intestine. They almost immediately hatch, liberating the rhabditiform larvae which are actively motile and are passed in the feces. (1) If the conditions are favorable in the area where the feces are deposited the larvae develop into free living males and females. If feces containing the rhabditiform larvae is allowed to stand for as long as 30 hours free living adult forms may be present. The females after reaching maturity and being fretilized by the male's oviposit about 50 to 75 embryonated eggs which hatch in a very short time liberating rhabditiform larvae. The larvae then undergo a period of development and after moulting become filariform in type. (2) If conditions are

unfavorable the rhabditiform larvae develop directly into filariform larvae. The filariform larvae are infective and if they come in contact with the skin of man or dog they will penetrate it and gain entrance to the blood or lymph stream. They are carried by the blood to the lungs and after a period of development break into the areolae, migrate to the bronchi, trachea, epiglottis, esophagus, and stomach to the jejunum where they develop into parthenogenic females.

Laboratory Diagnosis.

For practical purposes the finding of rhabditiform nematode larvae in the freshly passed feces of man is diagnostic.

Ancylostoma duodenale (Dubin, 1843) Creplin 1845.

This is the "old world hookworm". It is tropical and subtropical in its distribution and at times may be found in colder climates under special conditions, such as fecal contaminated mines, where moisture and temperature are favorable. The adults live in the jejunum and duodenum of man, occasionally in the pig and rarely in certain carnivorous animals. They are larger than the adults of N. Americana and have a less marked dorsal flexion of the head. This parasite is relatively uncommon in the United States and is only found in areas where immigrants from southern Europe live and take inadequate precautions in the disposal of human excrement.

Morphology.

The mature adults are small, plump, rigid round worms, yellowish white in color and the anterior extremity is slightly flexed dorsally. The buccal capsule or mouth is large, gaping and armed with six ventrally placed pointed dog-like teeth. The middle pair are small and difficult to demonstrate while the remaining four teeth are large, close together and conspicuous. The sub-ventral surface of the mouth cavity has two teeth-like lancets with rounded tips. The esophagus is club-shaped and measures about one-sixth of the length of the worm. It terminates posteriorly into the intestine which is a straight tube running caudally to the rectum. A muscular valve is present at the esophageal intestinal junction. A single, minute cervical papilla is present on each lateral margin of the cuticle, opposite the middle of the esophagus. The female worm measures 1.2 cm. in length and 600 M. in its greatest transverse diameter. The vulva is at the junction of the middle and posterior thirds of the body. The rectum is near the caudal tip. The generative organs consist of a pair of long curved tubes, one situated for the most part posterior to, and the other mostly anterior to the vulva. The ovary is the distal portion of each tube, the oviduct the middle portion and the proximal or terminal portion is the uterus. The uteri unite to form a common vagina which in turn opens into the vulva.

The male worm measures about 9 mm. in length and is about 500 M. in its greatest transverse diameter. The male genital organs consist of a very long coiled tubular testis which occupies the middle third of the body, a vas deferens, a spindle-shaped seminal vesicle, and a long ejaculatory duct opens into the rectum which is continuous with the cloaca

and copulatory bursa. Two protrusile long bristle-like copulatory spicules are situated in a sac-like structure on the terminal portion of the intestine. These copulatory spicules can be protruded into the copulatory bursa and are guided by a chitinous-like thickening called the gubernaculum. The copulatory spicules are not fused in A. duodenale, while in N. americana they fuse and the terminal portion of the fused spicule is armed with a fishhook-like barb. The copulatory bursa is made up of two wing-like projections at the caudal extremity of the worm and is supported by thick fleshy rays. The dorsal rays are tripartite and the clefts shallow, that of Necator americana bipartite and the clefts deep.

The eggs are oval in shape and have a colorless transparent shell. They measure about 60 by 35 M. and when observed in freshly passed feces contain a four to eight celled embryo. A clear space is present between the embryo and the shell. The eggs of A. duodenale, N. americana and A. braziliense are for practical purposes indistinguishable.

Life History.

The eggs as passed in the feces when deposited under favorable conditions on the ground develop into rhabditiform larvae, 250 M. in length within 12 to 24 hours. The larvae are actively motile and feed on organic matter at the edge of the fecal mass. At the end of 72 hours they become sluggish, the skin or cuticle is moulted and the larvae are 500 M. in elngth. Growth continues and the esophagus becomes filariform in shape by the end of 4 to 7 days. The larvae then moults again but does not cast its skin. The larvae enclosed in its old skin does not feed, is actively motile and may migrate as much as 4 inches of its own volition. The larvae are bluntly rounded anteriorly and their tails taper rapidly to a single point. Filariform larvae of S. stercoralis have a tail with a bifid tip. The larvae developed to this stage are infective and live a month if temperature and humidity conditions are favorable. In putrifying fecal matter in which conditions of anaerobiasis exist they quickly die. If the filariform larvae come in contact with the skin of man they penetrate it moulting the third time in the process, and gain access to the circulation, Where they are carried to the lungs. They then break through into the alveolae and migrate via the bronchi, trachea, epiglottis, esophagus and stomach to the intestines where they moult the third time and develop a small buccal cavity armed with 4 teeth. They attach themselves to the gut wall, grow to 4 mm. in length, moult the fourth time and then continue growing without further moults to the adult sexually mature worm. Fifteen to twenty days are required for the development time in the gut and five weeks from the time the filariform larvae enter the skin the adults are mature in the gut and passing eggs in the feces. A.duodenale females lay about 2 to 2½ times as many eggs per day as N. americana. It has been estimated that 28,000 eggs per day is the average output of one female.

Laboratory Diagnosis.

1. The finding of typical eggs in the freshly passed feces, followed by the recovery of typical adults in the feces after treatment.

Necator americana. (Stiles, 1902) Stiles, 1906.

This is the so-called new world hookworm although it in all probability was imported to the Americas by slaves from Africa during the colonization period. It is the most common helminth infestation in the United States where it is practically all limited to the area comprising the Southern States. Various surveys of this area have shown that 20 to 40% of the rural population are infested by this parasite. It is frequently found in tropical and subtropical regions in other parts of the world.

Morphology.

All hookworms are much like Ancylostoma duodenale. N. americana only differs from it in the following characteristics:

1. The average adults are slightly smaller.

	Length		Breadth	
	Males	Females	Males	Females
A. duodenale	9 mm.	12 mm.	500 M.	600 M.
N. americana	8 "	10 "	300 "	450 "

2. The head is only slightly dorsally flexed in A. duodenale and is markedly so in N. americana.

3. A. duodenale has two cervical papillae present on the cuticle at the level of the middle of the esophagus. They are absent in N. americana.

4. The buccal capsule is conspicuously large in A. duodenale and is armed with 6 teeth and 2 subventral lancets. The buccal capsule of N. americana has 4 needle-like subventral and two subdorsal lancets.

5. The vulva in the female, A. duodenale, is located at the juncture of the posterior and middle thirds. It is near the middle of the body in N. americana.

6. The dorsal rays of the male copulatory bursa are tripartite with shallow clefts in A. duodenale and bipartite with deep clefts in N. americana.

7. The male copulatory spicules are fused in their terminal portion and end with a fishhook-like barb in N. americana. They are unfused in A. duodenale and no barbs are present.

8. The eggs of N. americana are larger and narrower than A. duodenale, but for practical purposes are indistinguishable.

Life Cycle.

The same as for A. duodenale.

Laboratory Diagnosis.

1. Demonstration of typical eggs in the feces and recovery of adults in feces after treatment.

2. All cases of hookworm disease in the U. S. should be considered due to N. americana until proven otherwise.

Ancylostoma braziliense. Gomez de Faria, 1910.

This is the common tropical hookworm of dogs and cats. It is tropical and subtropical in its distribution. It is quite frequently found infesting these animals in the southern United States, but is a rare intestinal infestation in man. If the filariform larvae come in contact with human skin they will penetrate it and give rise to a local dermatitis known as creeping eruption. This skin condition is due to the migration of the larvae in the deeper layers of the skin, and the secondary bacterial infection that accompanies it. The larvae do not gain access to the circulation and probably never reach the intestines.

Ancylostoma caninum (Ercolani, 1859) Hall, 1913.

This is the most common hookworm of dogs and cats. It is cosmopolitan in its distribution and gives rise to a condition in dogs known as kennel anemia. The larvae may give rise to mild forms of creeping eruption in the skin of man but probably never infest his intestinal tract.

Wuchereria bancrofti (Cobbold, 1877)

This is the parasite responsible for most cases of elephantiasis and other types of localized parasitic lymph blockage. (However, only a small percentage of individuals infested with this parasite develop clinical elephantiasis.) W. bancrofti is tropical or subtropical in its distribution being particularly prevalent in certain sections of Africa and some of the Islands of the Pacific. It is found in Porto Rico, Panama, Philippines, Guam and in a limited area around Charleston, South Carolina and the coastal region between that city and Savannah, Georgia. The adult worms lie coiled in the lymph vessels just distal to lymph glands. When once developed they seem to be unable to pass or circumvent the lymph gland proper. Their mass and shape acts as an effectual plug in blocking the lymph vessels leading into the glands. This blockage is responsible for the lymph stasis and most of the pathological findings in this disease. The blockage may be acute with rapid swelling followed by sudden recession of the swelling due to movements of the worm opening the lymph channels. Ultimately the accompanying chronic inflammatory. reaction with scar tissue formation leads to chronic lymph blockage.

Morphology.

The adults are creamy white filariform worms with smooth cuticle, cylindrical shape and gradually tapering ends. The head end is knob-like

while the tail is bluntly rounded. The females measure 8 to 10 cm. in length by 0.24 to 0.3 mm. in breadth. The males 4 cm. in length by 0.1 mm. in breadth. The adults are extremely difficult to recover from the tissue. Attempts at extraction usually cause them to break into two or more pieces. They are best studied by using histological stained sections of tissue containing the parasites.

Life Cycle.

The females liberate the sheathed embryos into the lymph vessels. (The sheath of the embryo is not a retained moult but the stretched shell from the ova.) All females present, probably mature, and liberate their larvae simultaneously. The larvae measure 130 to 320 M. in length by 7.5 to 10 M. in diameter. The column of nuclei representing the primitive gut does not extend to the tip of the tail. The internal structures are undifferentiated. The larvae are motile and gain access to the peripheral circulation with a nightly periodicity starting at about 4 p.m. and increasing in numbers in the peripheral circulation until about 2 a.m. When periodicity is present few if any can be found during the day. In certain areas there is no periodicity or if present corresponds to the biting habits of the insect intermediate host. Nocturnal can be changed to diurnal periodicity by changing the sleeping habits from night to day time for two or three weeks.

If the larvae in the peripheral blood are ingested by suitable species of mosquito (Culex fatigans, Aedes variegatus, and Anopheles costalis incriminated to date) the larvae moult, migrate through the intestinal wall of the mosquito and eventually lodge in the thoracic muscles where they become quiescent, thick and short. After a period of a few days they develop their intestinal tract and generative organs. Growth continues, the cuticle is moulted and the larvae now about 1 mm. in length by 20 M. in breadth migrates from the thoracic muscles to the labium in which it lies. One to three weeks time are required for the mosquito cycle. When the mosquito again feeds the larva is dropped on the skin, and after penetrating it, migrates to the lymphatics and develops into an adult.

Laboratory Diagnosis.

1. The finding of sheathed nematode larvae with the column of nuclei not extending to the tip of the tail, in the blood, urine or chylous fluids of man.

2. Biopsy of lymphoid tissue with demonstration by histological means of the adults and larvae.

Dipetalonema perstans (Manson, 1891)

This is the filarial parasite the larvae of which have no periodicity in their appearance in the peripheral blood. It is found principally in the tropical sections of Africa and South America. The adults are found mainly in the retroperitoneal connective tissue and lymph channels.

Morphology

The adult females measure 7 cm. in length by 130 M. in breadth. The cuticle of both sexes is smooth when viewed microscopically. The heads of these species is crowned by two epalet-like appendages on the cuticle.

The larvae as seen in the peripheral blood are unsheathed and the column of nuclei extend to the tip of the tail.

Life Cycle.

The life cycle is the same as for W. bancrofti except that certain midges, Culecoides austeni and C. grahami are the insect intermediate hosts and vectors.

Laboratory Diagnosis.

The finding of unsheathed nematode larvae in the peripheral blood with a column of nuclei extending to the tip of the tail.

Filaria Ozzardi (Manson, 1897)

This filarial parasite is found in the West Indies and parts of South America. The life history and pathogenicity are unknown. The adults live in the retro-peritoneal tissue, abdominal wall and lymphatics. The whole adult male specimen has never been studied. The females are about 7 cm. long by 230 M. in breadth. The tail is terminated by a pair of large papillae. Unsheather larvae 200 x 5 M. without a column of nuclei extending to tip of tail are found in the blood stream. There is no periodicity in their occurrences.

Onchocerca volvulus (Leuckart, 1893)

This is the filarial parasite which is responsible for the development of nodular indurated swellings (calabar swellings) on the exposed surfaces of the body. These localized areas of swelling are most frequently found about the scalp and arms. They are due to the adult filaria nesting in the area and discharging their larva into the cutaneous lymphatics. The larvae are found in the cutaneous nodules but seldom if ever in the blood stream. This infestation is found principally in West Africa, South America and Central America. It is quite prevalent in the highland coffee plantation area of Guatemala and is a frequent cause of disfiguring face lesions and blindness. This is a chronic disease slow in progress but of long duration.

Morphology.

The females measure up to 34 cm. in length by 400 M. in breadth. The males 3 cm. in length by 150 M. in breadth. The cuticle of both sexes is raised in minute annular and oblique thickenings with serrated edges, which are more prominent towards the tail. The larvae are unsheathed, 350 t: 10 M. in size and do not have a column of nuclei to

the tip of the tail.

Life History.

The adult females liberate their larvae into the lymph spaces of the subcutaneous nodules. These nodules tend to ulcerate and are a favorite place for flies to alight and feed. The simulium flies are the intermediate hosts of this parasite. S. dammosum in Africa and S. mooseri and others in Central and South America. These flies do not feed on blood but bite and chew the superficial tissue ingesting the tissue, lymph, blood and serum exudate. In feeding on a nodule due to O. volvulus they also ingest the larvae. After a period of development in the simulium flie's gut and thoracic muscles the larvae migrate to the labium and are then infective if deposited on the skin by subsequent feedings of the fly.

Laboratory Diagnosis.

1. Smears from aspirated contents of skin nodules or skin scrapings of ulcers showing unsheathed nematode larvae 350 x 10 M. nuclei of tail not extending to tip.

2. Biopsy of nodules showing typical parasites.

Loa loa (Guigot, 1778)

This is the diurnal filaria parasite of West Africa and is rarely found in other countries except in people who have resided in West Africa and later migrated. The adults are peculiar in that they wander about the body and may be found in various tissues. They are most often seen making progressive motion under the conjunctival tissue of the eye. They are capable of moving in tissue at the rate of 1/2 inch a minute. The cuticle in both sexes is covered with small wart-like projections. The larvae are sheathed 200 x 10 M. in size and the nuclei of the tail extend into the tip.

The life cycle is the same as that described for W. bancrofti except that the chrysops flies are the intermediate hosts and insect vectors.

Dracunculus medinensis (Linnaeus, 1758)

This is the guinea worm and is found in the tropical and subtropical regions of India, Africa and South America. It is principally found in carnivorous animals in South America and to a lesser extent in man. The adults live in the subcutaneous connective tissue and fascial planes of muscle, chiefly of the arms, legs, ankle and foot.

Morphology.

The adult males are about 2 cm. in length but have only been found on two occasions. The adult females measure about 120 cm. long by 1.5 mm.

in breadth. The head has a chitinous shield and the vulva is situated very close to it. The tail is sharply bent into a hook shape.

The larvae are unsheathed, 700 x 25 M. and have a long tapering tail

Life History.

When the female is gravid and the larvae are ready to be liberated she approaches the skin surface on an extremity and causes an ulcer to form. When the ulcer is open and brought in contact with water the female protrudes her head and discharges a milky fluid containing myriads of embryos into the water. The embryos seek out the crustacean cyclops and develop in its body cavity to the infective stage. If the infected cyclops is ingested in drinking water the larvae escape in the intestines, invade the tissue and develop into adults. After fertilizing the female the male dies. The female then wanders in the tissue to one of the extremities where she comes to the surface and causes a surface skin ulcer.

Class Trematoda, Rudolphi, 1808.

The trematodes or flukes (except schistosomes in which the sexes are separate) are mostly parasitic hermaphroditic unsegmented, flat worms varying from 1 mm. in length to several centimeters. Two ventral prominent suckers are present near the head of all species and in the genus Heterophyes a third sucker is present around the genital pore. The digestive system consists of a mouth, esophagus and a bifurcated intestine which ends blindly. Anal openings are not present. The excretory system consists of flame cells, collecting tubules and excretory bladder. The male reproductive system consists of usually two various shaped testes (Genus Schistosoma 4 to 8), a pair of vas defferens and a vas defferens ending in a cirrus. The female genital organs consist of an ovary (usually fan shaped) a branched tubular oviduct, one branch of which ends blindly, the other in its subterminal portion is designated as the uterus and the terminal portion the vagina. The genital pores are inconspicuous and located just in front of or just behind the ventral sucker. The life cycle of these parasites involves two or more hosts with a definite period of development in each. For the trematodes of medical importance man is the definitive host while certain molluscs and sometimes crustaceans and fish act as intermediate hosts. There is always a period of parthenogenic multiplication in the mullusc while crustaceans or fish act only as carriers for encysted forms infective for man.

With the exception of Schistosoma japonicum in the Philippines and Schistosoma mansoni in Porto Rico and rarely Fasciola hepatica, the trematodes infections of man are not contracted and are seldom encountered in the U. S. or possessions. They will only be briefly discussed and demonstrated.

Definition of Terms

Cirrus - the male copulatory organ or penis.

Digenetic - Name applied to trematodes with a parthenogenic multiplication in the larval stages as well as the usual sexual multiplication in the adult stages.

Distomata - A term applied to trematodes with two suckers close together.

Gynaecophoric canal - The channel, formed by infolding of the lateral margins of the body in male schistosomes, in which the female lies.

Miracidium - The ciliated larva which develops in all trematode eggs and which is infective to mulluscs only. This is the first larval stage.

Sporocyst - The second larval stage occurring in mulluscs. It is sac or ribbon-like in shape and contains masses of germ cells. In schistosomes only, a sexual multiplication occurs in this stage.

Redia - this is the third larval stage and occurs only in molluscs. A sexual multiplication occurs in this larval stage of all species except schistosomes.

Cercaria - The final stage of larval development. It alone is infective for the definitive host (man, dog, cat, sheep, hogs, etc.).

Schistosoma mansoni, Sambon, 1907.

This is the blood fluke of Africa, the West Indies and South America. Porto Rico is the only United States territory in which it is found as a local parasite. The adults inhabit the hemorrhoidal plexus and the portal circulation of man. They are seldom found in other venous areas. They deposit their ova in the small venules of the rectum and rarely those of the bladder. The majority of pathological lesions due to this parasite are in the rectum or liver. These lesions are typified by chronic inflammation, granulation, fibrosis, ulceration and scar tissue contractions. Numerous large spindle shaped eggs with lateral spines are found in or on the inflammatory tissue, some are liberated into the rectum, rarely into the bladder and others undergo degeneration and calcification in the tissue. Diarrhea, ulcerative proctitis and hepatitis with ultimate cirrhosis are frequent complications of this disease.

Schistosoma haematobium (Bilharz, 1852) Weinland, 1858

This is the blood fluke causing Bilharz disease, which is characterized by haemature, cystitis, urethritis and hepatitis with cirrhosis. It is found in parts of Africa, Arabia, Palestine, and Mesopotamia. The adults inhabit the portal vein and its radicles and especially the vesical plexuses of man. The large broadly spindle shaped eggs with posterior terminal spine are found usually in the urine, rarely in the feces. This disease has never been known to have been contracted in the United States or its possessions.

Schistosoma japonicum, Katsurada, 1904

This is the blood fluke causing schistosomiasis in Japan, China and certain areas in the Philippine Islands. The adult worms are found in the small veins of the large intestine particularly in the hemorrhoidal plexuses, but may occur in the gastric, mesenteric and other veins. Man, horses, cattle, pigs, dogs, cats, and experimentally mice may be infested by this parasite. The eggs are large, oval to spherical in shape with a small know-like projection on one side which is sometimes hard to demonstrate. The eggs are found in the feces, ulcerative lesions of the colon and rectum and in the liver. The disease resulting from infestation with this parastie is characterized by diarrhea, colitis, and practitis followed by cirrhosis of the liver, ascites and edema.

A detailed description of the morphology and life cycles of the schistosomes will not be given but will be summarized in the following table:

Males

	Length	Breadth	Type of cuticle	Number of testes
S. haematobium	1 cm.	1 mm.	Fine tuberculated	4
S. mansoni	1 cm.	1.3 mm.	Coarse tuberculated	8
S. japonicum	1 cm.	500 M.	Smooth	8

Females

	Length	Breadth	Position of ovary in body	No. of ova in uterus	Ova deposited in
S. Haematobium	2 cm.	250 M.	Posterior $\frac{1}{2}$	20:30	Singly
S. Mansoni	12:16 mm.	170 M.	Anterior $\frac{1}{2}$	1:3	Singly
S. Japonicum	2 cm.	400 M.	Middle	30:50	In masses (10:30)

Ova

	Shape	Where found	Length	Breadth
S. haematobium	Spindle shaped Terminal spine	Urine Rarely feces	120:160 M.	40:60 M.
S. mansoni	Spindle shaped Lateral spine	Feces	140:165 M.	60:70 M.
S. japonicum	Broadly oval with small subterminal knob on surface	Feces	70:100 M.	50:65 M.

Fresh water molluscs acting as intermediate hosts.

S. haematobium - Bulinus truncatus, Physopsis africana, P. globosa and Planorbid dufouri.

S. mansoni - Planorbis boissyi, P. pfeifferi, P. olivaceus, P. guadaloupensis.

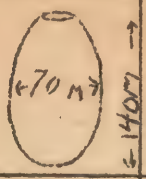
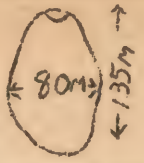
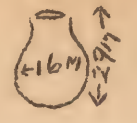
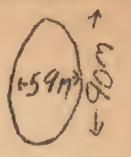
S. japonicum - Oncomelania noxophora, O. formosana, O. hupensis.

A representative life cycle for the schistosomes may be summarized as follows:

The eggs as passed in the urine or feces contain a ciliated larvae called a miracidium. When the eggs come in contact with water they hatch liberating the ciliated miracidium which swims rapidly about searching a suitable mollusc host. If it does not find one within a few hours it dies. When the miracidium finds its mollusc host it penetrates the tentacles and losing its cilia, and other special organs for which it has no further use, it migrates to the visceral mass and becomes a sporocyst. The sporocyst multiplies by formation of daughter sporocysts until by the end of a few weeks the visceral mass of the snail is permeated with these minute, elongated, delicate, tube-like larvae. Five to seven weeks after infesting the snail the sporocysts stop producing further progeny and develop into the final larval form, the cercaria, which are infective to man. The cercaria are forked tailed, glider-like larvae and are capable of swimming rapidly. They will swim toward anything agitating the water such as a man wading or swimming. Upon gaining contact with the skin of man they penetrate it, shed their tails, gain access to the circulation and are carried to portal circulation by the blood stream where they develop into adults within a period of three weeks. When mature the females enter the male gynaecophoric canal and remain there in copulation until ready to oviposit. When this time arrive the females leave the males and work their way against the blood currents until they reach a small venule in the hemorrhoidal or vesicle plexus. They force their way into a vessel that is slightly smaller than their transverse diameter by stretching it, deposit their eggs and withdraw. The eggs with spines are deposited so that the spines face the direction of the blood flow. The blood pushing against them force the spine into the vessel wall which soon causes a pressure necrosis and rupture into the feces or urine. S. japonicum eggs are deposited in a mass blocking the venule, this leads to thrombosis, necrosis and ulceration with discharge of the ova into the feces.

A summary of the life history of other flukes that may be encountered in man in the United States or its possessions is given below:

Summary of Life History of Flukes Infesting Man

	F.hepatica	F. buski	C.sinensis	P.westermani
Egg:-				
Egg contains miracidium when passed in:-	Feces	Feces	Feces	Sputum
Egg reaches fresh water and the miracidium escapes, swims about and invades a mollusc:-	Snail (Limnaea truncatula).	Snails (Planorbis schmackeri, Segmentina Nitidellus)	Snails (Bithynia Spp.).	Snail (Melania libertina).
Miracidium loses its cilia and special organs & becomes sporocyst. Migrates to visceral mass of mollusc	Yes	Yes	Yes	Yes
Sperm cells develop within the sporocyst & produce rediae:-	Yes	Yes	Yes	Yes
Rediae product daughter rediae inside themselves; these escape via birthpore & permeate visceral mass of the mollusc:-	Yes	Yes	Yes	Yes
Mature rediae develop within themselves cercariae, these likewise escape via birthpore to visceral mass of mollusc:-	Yes	Yes	Yes	Yes
Cercariae leave snail & swim about in water, enter second host of larva which is:-	No second larval host cercariae encyst on grass.	No second larval host cercariae encyst on water plants	Fresh water fish, various species.	Fresh water crabs and crayfish.
Definitive host:-	Sheep, rarely man.	Man and pig.	Man, cats & dogs.	Man, dog, cat, tiger wolf, pig.
Definitive hosts eats and chews:-	Grass containing encysted larva.	Water plants containing encysted larva.	Raw fish	Raw crabs or crayfish.
The live cercariae thus swallowed, each become adults in the:-	Bile ducts	Small intestine.	Bile ducts	Lungs

Feces.

1. Examination for adult helminths:

A careful inspection of the fecal specimen will disclose any large helminth present as Ascaris lumbricoides or the large tapeworms. It will be difficult, unless great numbers are present, to demonstrate by such a procedure, small helminths, such as hookworms, pinworms, or the dwarf tapeworm. For this type of worm a method of concentration should be used. The procedure is as follows; secure a large specimen of feces and dilute it with tap water until a thin solution is obtained. The fecal solution should then be strained through wire screen or gauze to get rid of the fecal matter, any small helminth present will be retained on the strainer. Wash the material retained on the strainer with additional amounts of tap water and transfer it to a shallow flat bottomed white enameled pan. The transfer is best done by inverting the strainer over the pan and then washing the material into the pan with about 100 cc. of tap water. A cotton applicator or long handled camel's hair brush is then used to go over the material bit by bit to detect any helminth present.

2. Examination for larvae:

A small bit of feces is selected and emulsified in a drop of water upon a glass slide. A cover slip is applied to the emulsion and the preparation examined under low power magnification. The amount of feces used in making the preparation should be such that when the cover slip is applied ordinary news print can still be vusualized through it. Examine the specimen carefully, if Strongyloides larvae are present they can be easily found due to their active movement and the commotion created by them.

3. Examination for eggs:

a. Non-operculated eggs.

(1) Direct smear method. Examine by the fresh wet preparation the same as for larvae. If Schistosome eggs are suspected select a bit of bloody mucus if present.

(2) Concentration method. (Should be routine on all specimens)

(a) Reagent.

25 per cent sucrose (cane sugar) solution in tap water, to which is added 0.5% phenol as a preservative. Make up 1000 cc. at a time and keep on hand for routine use.

(b) Equipment.

(1) Test tubes 16 x 150 mm. (standard bacteriological culture tubes).

(2) Applicator sticks.

(3) Steel wool.

(4) Wire loop, loop to be 1/4 inch in diamater and bent at right angles to the shaft of the wire.

(5) Glass slides and coverslips.

(c) Procedure.

Select a piece of feces the size of a hazel nut and place it in the bottom of a test tube. Add 2 cc. of the sugar solution and thoroughly emulsify the feces by means of an applicator stick. When the feces is liquid in consistency, fill the tube to within one-eighth inch of the top with additional sugar solution. If there is much floating debris drop a small piece steel wool into the tube t sink it. Let the specimen stand in a vertical position for 30 minutes. Using the wire loop remove the uppermost layer of the solution and place it on a glass slide, coverslip it and examine.

b. Operculated eggs.

Use the fresh wet preparation method and the concentration method given for Protozoan cysts.

c. The Stoll egg counting method. (Used in estimating the number of helminths present by the egg count of the feces).

(1) Method.

Weigh 5 grams of feces into a large test tube graduated at 75 cc. Fill the test tube to the 75 cc. mark with N/10 NaOH (4 grams of NaOH to 1000 cc. of water is sufficiently accurate). Add several glass beads, close the tube with a rubber stopper and shake until a homogeneous solution is obtained. Transfer 0.15 cc. of the suspension to a 2 x 3 inch glass slide and coverslip it with a number 2. coverslip (22 x 40 mm.). Count the number of eggs present, the average of two such counts should be the count selected.

(2) Interpretation.

(a) Counts on liquid and mushy stools must be converted to correspond to formed stool amounts.

(1) To convert mushy stool counts to formed stool averages multiply by two.

(2) To convert liquid stool counts to formed stool averages multiply by four.

(b) When formed stools are used in making up the suspension the count of a 0.15 cc. sample of the mixture multiplied by 100 gives the number of eggs per gram of feces.

(c) 44 eggs per gram of feces indicates one female Necator americana present.

Blood.

Ova are not found in the blood. Larvae present are best demonstrated by (1) thick blood films made at the optimum time, stained with Giemsa stain; (2) Drawing of 10 or more cc. of blood at the optimum time, laking it with distilled water, centrifuging the laked blood and examination of the sedimented residue; (3) if great numbers of the larvae are present they may be demonstrated by making wet mounts of the blood and examining the preparation direct without staining it. Most larvae are actively motile and can be easily seen under low power magnification.

Urine.

Filaria larvae or Shistosome ova are the only helminth material likely to be encountered. They are best demonstrated by examining the centrifuged sediment of a specimen of urine.

Chylous fluid.

Filaria larvae may be present and are best demonstrated by examining the sediment of a centrifuged specimen.

Sputum.

Examine by: (1) The wet mount method selecting a blood tinged portion if available. (2) Add four volumes of N/10 sodium hydroxid , thoroughly mix and allow to digest for 2 to 4 hours, centrifuge and examin the sedimented residue.

Skin.

1. Examination for eggs.

The only eggs likely to be found are those of pinworm. They are most likely to be detected in swabbings from the perianal region. Hall's method is the best available and is as follows:

a. Material

(1) Light weight cellophane (similar to that used on cigarette packages) cut into one inch squares.

(2) One-eighth inch glass rod cut in 4 inch lengths and fire polished on each end.

(3) Glass vials 10 x 60 mm. with a one hole rubber stopper to fit.

(4) Rubber bands made by cutting 1/8 inch pieces from 1/8 inch rubber tubing.

b. Assembly.

Insert a glass rod through the rubber stopper of a vial so that it comes to within 1/4 inch of the bottom. Remove the stopper containing the glass rod from the vial and fold a square of cellophane over the end which goes in the vial. Secure the cellophane in place with one of the small rubber bands. Replace the prepared swab into the vial and it is ready for use.

c. Securing the specimen.

The specimen should be secured from the patient the first thing in the morning before he has had the opportunity to take a bath or defecate. To secure the specimen remove the swab from the vial and swab the perianal region with the cellophane end. Replace the swab back in the vial, label and send to the laboratory for examination.

d. Examining the specimen.

Place a drop of N/10 sodium hydroxide on a clean glass slide. Remove the swab from the vial, holding it vertical place the cellophane end into the drop NaOH. using a forcep slip the rubber band up the glass rod and then straighten out the cellophane so that it is like a coverslip on the drop of NaOH. Remove the rod, place an additional drop of NaOH on top of the cellophane and apply a coverslip. Examine the preparation under low power magnification.

2. Examination for larvae.

Onchocerca larvae may be present in skin nodules and can be demonstrated by studying aspirated lymph, tissue scrapings, tissue juice or by making stained histological sections.

Other tissue.

The demonstration of helminth parasites in tissue is done by: (1) histological study of biopsy material. (2) Digestion of the tissue with 10 to 20 volumes of a solution containing 0.5% pepsin and 0.7% hydrochloric acit. Followed by concentration of the larvae present by using the

Baermann apparatus. (3) Direct examination of the tissue by pressing it into a thin layer between two heavy glass slides. All of the above methods are of value in demonstrating trichina larvae.

Soil.

1. Eggs.

Soil contaminated with helminth eggs such as Ascaris is best examined by the floatation concentration method given under feces, 50 to 100 gram samples of solid should be used.

2. Larvae.

The recovery of helminth larvae from soil is best done by using the Baermann apparatus. Large samples of the soil being studied should be used.

Serological Diagnosis.

This means of diagnosing helminth infections is as a rule unsatisfactory. It should only be used when other methods are not applicable. It should only be done in large laboratories where special precautions can be taken to insure satisfactory antigens and controls.

The Baermann apparatus.

It consists of: (1) A glass funnel 6 to 10 inches in diameter fitted with a short piece of 1/4 inch rubber tubing and a Hoffman clamp. (2) A round basket 4 to 8 inches in diameter and 3 inches deep made of 1 mm. wire screen. (3) Cheese cloth or gauze.

The sample of soil or digested tissue to be tested is placed in the cheese cloth lined basket and the basket suspended in the funnel. Water heated to 100 degrees F. is run into the funnel until its level just touches the bottom of the basket. The material is allowed to stand and after a period of 1 to 4 hours, live larvae if present will be found in the water in the lower portion of the funnel's stem. A piece of ice placed on top of the specimen will hasten the migration of the larvae. The larvae are observed by either drawing of a small portion of the water and examining it direct under the microscope or by drawing of a 10 cc. sample, centrifuging it and then examining the sediment.

Shipment of helminth material for diagnosis at a central laboratory.

1. Specimens of large helminths.

Kill and fix the specimens in hot (85) degree Centigrade) 5 per cent formalin in tap water. Select a suitable container and ship the specimen in a solution of 5 per cent formalin and 2 per cent glycerin tap water.

2. Fecal specimens, larvae or eggs.

(a) Fixed specimen.

Use at least two volumes of hot (85 degree Centigrade) 5 per cent formalin in tap water for fixation and then ship in a suitable container.

(b) Unfixed specimen.

When the distance which the material will have to be shipped is not too great an unfixed specimen should always be shipped If possible pack the specimen bottle in a container with a small amount of dry ice.

(c) Specimens on glass slides such as filaria smears take adequate precautions to prevent breakage.

File: 222 revised

HEMATOXYLIN STAINING TECHNIQUE FOR FECES

For fixed preparations a smear is made with a paste brush on a clean flamed slide. If the fecal material is too dry, moisten slightly with normal saline. If it is too liquid, smear a drop of egg albumen on the slide first, allow to dry slightly, and then brush over with specimen. Without allowing the slide to dry immerse it immediately in fixing fluid.

SOLUTIONS

1. SCHAUDINN'S FLUID:

   a. Stock solution:

Mercury bichloride, saturated solution in normal saline (saturation is 5.73 grams per 100 cc.)	2 parts
Alcohol 95 per cent	1 part

   This solution will keep indefinitely.

   b. Add 2 cc. glacial acetic acid to 48 cc. of stock solution.

   This solution is unstable and should be made up fresh before use and discarded immediately afterwards.

2. HEMATOXYLIN STAIN:

   a. Stock solution 1:

Hematoxylin	5 grams
Distilled water	1000 c.c.

   Place in clear glass bottle, cork and ripen in a light place for 1 month or longer.

3. CARBOL XYLOL:

Xylol	3 parts
Liquefied Phenol (Merk's Blue label grade)	1 part

4. MORDANT:

Ferric ammonium sulfate	2 grams
Distilled water	100.0 c.c.

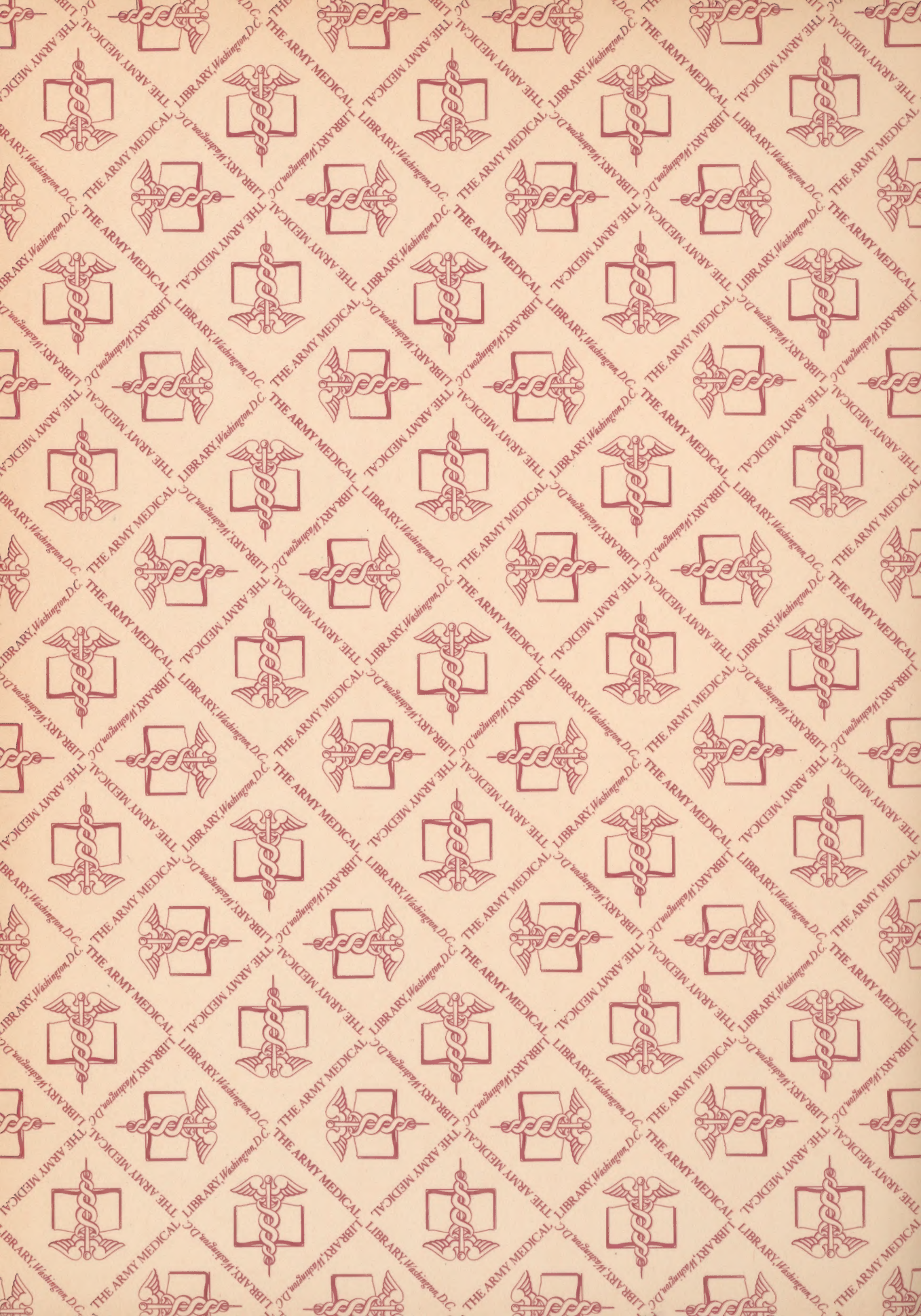
5. DECOLORIZER:

Ferric ammonium sulfate	0.5 gram
Distilled water	100.0 c.c.

METHOD

Fix wet smears in Schaudinn's fluid, heated to 60° C. in water bath	30 min.
(Schaudinn's fluid may be used cold - 8 hours)	
70% Alcohol tinged to wine color with iodine	5 min.
70% Alcohol	5 min.
50% Alcohol	5 min.
Wash in running tap water	2 min. (or more)
Mordant (2% Ferric Ammonium Sulfate, heated to 40° C.)	10 min.
Wash in running tap water	10 min.
Hematoxylin stain, heated to 40° C.	10 min.
Wash in running tap water	5 min. (or more)
Decolorizer (0.5% Ferric Ammonium Sulfate)	5 min.
Wash in running tap water for a minimum of -	10 min.
Graded alcohols - 50% - 70% - 95%	5 min. each
Absolute alcohol	5 min.
Carbol-Xylol	5 min.
Xylol	5 min.
Mount with a thin layer of balsam, thinned with Xylol, and cover slip.	

NOTE: The decolorization process is quite variable. So make more than 1 (one) slide and after decolorizing for 5 minutes, take 1 of the slides and rapidly run through the alcohols and xylene solutions leaving it in each for about 10 seconds. Mount the slide with balsam and a coverslip and examine under the microscope. Rarely, if ever, will the slides be too light; if so, place in the stain again and decolorize less than 5 minutes. Usually the slides will be just right or a little dark. If too dark, decolorize for another minute and test the slides again.


THE ARMY MEDICAL LIBRARY, Washington, D.C.